

That Was the Debate, That Was

THE great argument about the British government's policy for civil research in Britain has turned out to be more noisy than seemed at first to be likely (see *Nature*, 234, 240; 1971), but it has not been much of a debate. The scientific community has, to its credit, spoken out, sometimes with expressions of fear, rational and irrational, sometimes with reasoned and valid arguments why the Rothschild recipe in its starkest form is inapplicable and sometimes with constructive suggestions. Throughout the time allotted for the debate, the government has been silent. Only Lord Rothschild has intervened from time to time to emphasize some parts of his case for a radical revision of present arrangements or to dot the i's and cross the t's in his original declaration (see page 296). It is true, of course, that the government has endorsed the customer-contractor principle, although nobody at this stage would regard that as more than an expression of hope and an affirmation of faith in the way in which governments should intervene. As a result, it is still far too early to tell what the practical outcome of recent clamour will be. The most ominous portent is that the government departments have by all accounts been staking out as large a claim as they can for as large a share of the spending power of the research councils as possible. In the circumstances, the greatest danger is that the Rothschild recipe will be used as an excuse for carrying through a premature and ill-judged reorganization of the present system.

To say this is not to deny the value of Lord Rothschild's essay in organization or even the usefulness of the customer-contractor principle. Much of the protest against the Rothschild recipe in the past few weeks has consisted of arguments that it is impossible to pre-determine the outcome of research of the type at present sponsored by the research councils so as to answer the questions which customers ask, but in some fundamental sense this is quite beside the point. The essence of Lord Rothschild's case is that in advanced societies such as Britain, taxpayers and the governments which represent them should play a more active and direct part in plotting the strategy for scientific research. In other words, the customer-contractor principle should have brought joy to those who have been urging in recent years that science should be more socially responsive (which is why it is a little odd that the British Society for Social Responsibility in Science has ranged itself in opposition to Lord Rothschild's proposals). Moreover, it is clear that there are many urgent problems to which publicly sponsored research could make valuable contributions. Is there not, for example, a case for more pointed research on city planning, the development of medical services in Britain, the equipping of British farmers with the methods (and the seeds) best suited to the European Communities, the exploration for minerals and the development of economical methods of extracting them, and the development of more efficient ways of teaching children? To say, as the common defence of

the research council system implies, that all these benefits will in due course emerge, if only the research councils are allowed to continue as they have done for the past decade, does nothing to meet the charge that society at large through the instrument of the British government must have a say in determining objectives for applied civil research and in setting a time scale. At this level, Lord Rothschild's recipe is a constitutional point which is crudely misrepresented by the slogan that "He who pays the piper calls the tune". Moreover, it is undeniable.

The example of the United States is in this connexion relevant, and it is no accident that one of the most telling contributions to the debate that never was is that of Dr Harvey Brooks (see page 301). The truth is that the United States has since the Second World War used the customer-contractor principle for supporting a large part of applied research in the civil field. In the decades since the war, indeed, the chief complaint against the planning of scientific research in the United States has been that the customer-contractor principle, embodied in the letting of research and development contracts by government agencies, has dangerously distorted the system and in particular has so starved the National Science Foundation of funds that the long-term development of university research has been impeded. It is also true, of course, that many American customers have asked foolish questions of their research contractors—many of the projects undertaken by NASA, the biosatellites and Earth resources satellites, for example — were misguided attempts to squeeze blood out of a stone in the sense that there were no grounds on which the fond hopes for these projects could be justified. But that only goes to show that customers often let their hearts get the better of their heads, and it is lamentable that the National Science Foundation, still inadequately supplied with funds for supporting research in the educational sector, should have been compelled to jump on the bandwagon with its programme of Research Applied to National Needs (RANN). At the same time, as Dr Brooks points out, the customer-contractor principle has had some remarkable and beneficial results in the United States. The lesson which should be learned in Britain is that there is nothing wrong with the principle but that, like all principles, it can be misapplied.

The second strong argument in favour of Lord Rothschild's recipe is that it could well serve as a way of strengthening British government departments scientifically. Over the years, one of the constant complaints against the British Civil Service has been its neglect of science in its higher counsels. The fact that the research councils have been so far largely responsible for the development of strategy in an important sector of the country's applied research is a measure of the government's failure to have a mind of its own. If some measure of direction is now to be provided by the departments, and if the Civil Service takes to heart the warnings



in Lord Rothschild's own proposals that the customer-contractor principle will work only if government departments are properly equipped to ask intelligent questions of their potential contractors, the result should be that government departments are much better provided than in the past with a sense of what applied research can accomplish for the public good. But this, of course, will be a hard lesson for many departments to learn. The most serious danger is that they will set out to let research contracts without providing themselves with the skills necessary for the proper conduct of their new responsibilities. The portents of the past few weeks have not been entirely encouraging.

Of the arguments against the Rothschild recipe, those which carry most weight concern practice and not principle. In the rumblings of the past two weeks, it tends to have been forgotten that the Rothschild report itself asks only that a maximum of one-eighth of the present spending of the research councils should be transferred to the relevant government departments in the course of the next few years, but even so, there are dangers that in the process of transferring responsibility for expenditure serious damage might be done to the largely successful methods by which the research councils at present function. For one thing, there are risks that an excessively hamfisted approach by government departments might undermine the freedom or the sense of freedom at present enjoyed by many of those who work as professional scientists for the research councils. The consequence might be seriously to diminish the willingness of professional scientists to work in the public service (or even in Britain) and the loss of confidence and of people could far outweigh the benefits to be derived from the application of the customer-contractor principle. It follows that any reorganization on which the government now embarks should include provisions for strengthening the sense of autonomy of which the research councils have been jealous—and rightly jealous—in the past few years.

For one thing, they should be enabled to determine policy in the areas for which they remain responsible with even greater independence from the Department of Education and Science than in the past few years. In particular, the system by means of which independent professional scientists from all walks of life are recruited into the decision-making process through the research council committees, which has served as a powerful means of giving the scientific community a sense of community (and which is well worth the administrative cost which Lord Rothschild begrudges), should be preserved. Second, the research councils should be told quite plainly that in their role as potential contractors for government departments, they should be free to negotiate the terms and even the objectives of contracts with all the independence that commercial contractors enjoy. (It goes without saying that in such a relationship, both the research councils and the government departments would be surprised to see how expensive research projects become once they are designed so as to produce results to order.) In such a setting, there is plainly room for a strong board to oversee the operation of the research councils, and this is the point at which Sir Frederick Dainton's alternative framework for policy-making has an important part to play. There is, after all, no fierce inconsistency between the two

sets of proposals and there is no reason why the Dainton proposals should not be implemented in parallel with whatever use is made of the customer-contractor principle.

These are the lines on which the government should seek a resolution to its present dilemma. First of all, it should reaffirm its belief that in the long run the customer-contractor principle has an important part to play in the organization of applied research. It should then acknowledge that there are important practical problems to be solved before the principle can be applied even in the comparatively small part of the field now covered by the research councils on which Lord Rothschild has set his sights. In the circumstances, there is an urgent need to test the mechanism by which these arrangements, novel for Britain, would be applied, so that it would be wrong to commit the research councils or the government departments to a cut and dried timetable for the transfer of financial responsibility from one to the other. Instead, the government should identify some area of applied research which is urgent and important in which deliberate attempts might be made to make the customers intelligent and the contractors responsive. The government will not merely earn credit among scientists but gratitude from its taxpayers if it begins by saying that it has no predetermined commitment to some arbitrary timetable.

Obviously there would be advantages if the first steps in the application of the customer-contractor principle consisted of attempts to strengthen applied research in areas which are at present neglected. Would it not be possible, for example, for the first contracts to be ones that seek to use applied mathematics and civil engineering for the better design of cities, or modern techniques in education for the improvement of the medical curriculum and the development of methods of teaching it? It is not, after all, as if Britain has too much applied research. At the same time, the government should go a long way to implement the constructive proposals in the Dainton report and in particular to set up a grand co-ordinating board (without the *ex officio* membership of the president of the Royal Society) not merely to hold the ring between the research councils but also to follow Sir Frederick Dainton's advice that this body should, where necessary, create new research councils or put those which become defunct to sleep.

There remains the most serious weakness of the Rothschild recipe (not Lord Rothschild's responsibility)—that the present argument is concerned with only a tiny part of the British government's sponsorship of applied research. In retrospect, as at the time of publication, it is absurd that so much energy should have been spent on proposals for reorganization which leave quite untouched the work already sponsored directly by government departments and especially the work of the large government research establishments now under the Department of Trade and Industry and the Ministry of Defence. For the truth is that if the research councils are to be brought within a framework for permitting the government to settle priorities in applied research, it is essential that the great fiefdoms of the Atomic Energy Authority and the Ministry of Defence should be made a part of the same organization. The suspicion remains that the government has chosen not to tackle this part of the problem of applied research in Britain because it recognizes the power

of vested interests. Yet its duty is to say quite plainly that during the period when modest and tentative initiatives will be made in applying the customer-contractor principle to the research councils, it will institute the careful study of the whole of applied research which is necessary if the fullest use is to be made of the country's scientific power.

Forward by Millimetres

THE British government is keeping its head low on metrication. The white paper on the subject published last week (see page 297) confirms the previous government's declaration to encourage metrication in Britain and to work to a timetable that will see a large part of the changeover completed by 1975, but it does so without the enthusiasm with which the old Ministry of Technology embraced the prospect of this change. The reasons, of course, are simple enough. For one thing, the present government's supporters in the House of Commons are lukewarm on the subject. Then the government is having to live uncomfortably with the legacy of decimalization bequeathed to it by Mr James Callaghan on a system which has brought an unpalatable degree of price inflation in Britain.

No wonder that the government and the Department of Trade and Industry is anxious to keep its head down. No wonder that it accompanies the general advice that metrication is a necessary part of membership of the European Communities with declarations that "it will be a long time" before road signs are changed and that it will be possible for the indefinite future to buy beer and milk by the pint and not the litre. The trouble, of course, is that if metrication is worth having at all, it will need to be encouraged and that this will be possible only if the government makes such a firm commitment to the principle that the Metrication Board can function efficiently.

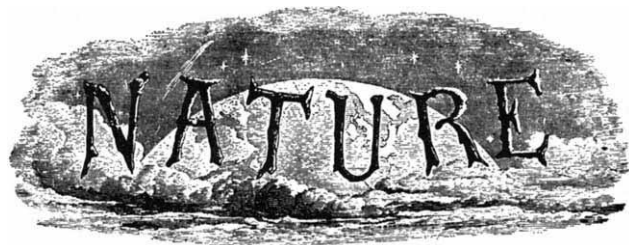
One of the most obvious gaps in the document now published is its qualitative character. The white paper makes a great deal of the way in which several industries, urged on by the Confederation of British Industry, have voluntarily made the change. In practice, the pharmaceutical industry was understandably one of the first to adopt metric units, but that was a natural development partly because of the international character of its business and partly because British pharmacists were lumbered with a system of weights and measures even more anachronistic than other parts of commercial life. It was something of a surprise that the construction industry in Britain, not conspicuously the most advanced, should have been an early starter, but this is a field in which the standardization of modular sizes has forced the pace. But in the circumstances, it might have been expected that the Department of Trade and Industry could now have had enough experience to spell out in some detail not merely the costs of conversion but also the potential benefits.

The white paper explains that such calculations are complicated by the way in which a decision to convert to metric measures within a factory or an industry is often accompanied by more far-reaching decisions to rationalize a line of products or in which the timing of metrication is

chosen so as to fit in with other changes of organization within a company, and it is true that complications like these must necessarily make difficult the calculation of costs. Even so, it would have been helpful to know more about the ways in which the companies which have made the change have found it necessary to lay out money. And what experience has there been on the cost of training people to use unfamiliar units? The fact that it has been hard to quantify experiences like these is, after all, interesting in itself, for it may well be that the difficulties are not nearly as formidable nor as costly as some critics fear they are.

What are the potential benefits? The starting point for objective calculation should be, of course, the experience of those salesmen who are still required by their employers to sell goods abroad in sizes quoted in imperial units. The white paper points out that 15 per cent of the Gross National Product of Britain is at present represented by exports and, although a large part of the export trade is with the United States, there is no question that a large part of the present export business will disappear if metrication is not quickly implemented. And the diversity of the export trade is in itself a proof that the change cannot be confined to a narrow sector of British industry. In the circumstances, it would have been more hopeful if the white paper had contained a more explicit timetable for the change. It should also have had more to say about the ways in which the change could be encouraged in the schools and about the zeal with which it will urge that change on.

100 Years Ago



WE have reason to know that many weak people have been alarmed, and many still weaker people made positively ill, by an announcement which has appeared in almost all the newspapers, to the effect that Prof. Plantamour, of Geneva, has discovered a comet of immense size, which is to "collide," as our American friends would say, with our planet on the 12th of August next. We fear that there is no foundation whatever for the rumour. In the present state of science nothing could be more acceptable than the appearance of a good large comet, and the nearer it comes to us the better, for the spectroscope has a long account to settle with the whole genus, which up to this present time has fairly eluded our grasp. But it is not too much to suppose that the laymen in these matters might imagine that discovery would be too dearly bought by the ruin of our planet. Doubtless, if such ruin were possible, or indeed probable—but let us discuss this point. Kepler, who was wont to say that there are as many comets in the sky as fishes in the ocean, has had his opinion endorsed in later times by Arago, who has estimated the number of these bodies which traverse the solar system as 17,500,000. But what follows from this? Surely that comets are very harmless bodies or the planetary system, the earth included, would have suffered from them long before this, even if we do not admit that the earth is as old as geologists would make it.

From Nature, 5, 310, February 15, 1872

OLD WORLD

Dainton Demands a Hearing

SIR FREDERICK DAINTON, in evidence before the Select Committee on Science and Technology, last week welcomed the extension of the customer-contractor principle "where relevant", but he added that the Council for Scientific Policy does not accept the scale, method of implementation or timetable proposed by Lord Rothschild.

In a memorandum to the Select Committee, the Council for Scientific Policy stated that Lord Rothschild's proposals for the adoption of the customer-contractor principle "will lead to a situation which would seriously handicap the scientific resources of this country by making it more difficult to determine

coherent policies for scientific research as a whole". The memorandum also pointed out that the implementation of the principle on the scale suggested by Lord Rothschild would limit "the basic and strategic research which the research councils could undertake on their own initiative in their own establishments and in conjunction with the universities".

In contrast to Lord Rothschild, who holds the diametrically opposite view, Sir Frederick did not consider the research councils to be monopolistic. The research councils, said Sir Frederick, produced annual reports and thus they made themselves accountable to parlia-

ment. Sir Frederick also said that although the research councils receive their money from one department only—the Department of Education and Science—the present system of accountability is adequate, in particular as parliament controlled the purse strings.

Possibly as a sop to Lord Rothschild and to the government, which has endorsed the customer-contractor principle, the Council for Scientific Policy in collaboration with the research councils has identified £7 million of applied research at present in the research councils' budgets that could be transferred to the departments. The CSP, however, makes it clear in its memorandum that no transfer of funds should take place until the departments have set up efficient chief scientist organizations. The sum it recommends, however, is much less than the £27.7 million that Lord Rothschild proposes should be transferred in Table 4 of his report. The CSP says that this sum "is entirely wrong" and that it was produced "without any discussion with the councils about its composition and the explanation of its provenance is unconvincing".

The Council for Scientific Policy welcomes the government endorsement of the research council system and although it is in broad agreement with Lord Rothschild when it says that tactical research should "be regarded as potentially susceptible to the customer-contractor principle" it reaffirms that basic research and strategic research should continue to be done by the research councils. Sir Frederick was concerned that the research council system which was set up so recently, and against which no serious criticisms had been raised, would be seriously debilitated by Lord Rothschild's proposals. The CSP recommends that the research councils should be involved more in the work of the departments by encouraging the departments to place more contracts with the research councils. Sir Frederick said that he thought it was quite possible for the departments to contract £20 million to £30 million of their total research and development budget of £536 million to the research councils.

Sir Frederick completed his report in May 1971, two months after Lord Rothschild started work on his study, but the report was not published for a further six months, in spite of Sir Frederick's request for quick publication. The consultations of the Council for Scientific Policy were restricted to the Department of Education and Science because when the CSP was

Self Evident—an explanation by Lord Rothschild

THE last sentence in the article entitled "Lord Rothschild in the Dock" in *Nature* (235, 240; 1971) states that in my evidence to the Select Committee on Science and Technology "He felt that a great deal of the misinterpretation had arisen because he had not been too specific, and that a few extra paragraphs to qualify his point of view would have been worth while". This sentence bears little relation to what in fact I said in this context, which was "If I could put the clock back, I think the mistake in my report is that it is too short. I would like to multiply its length by a factor of ten at the risk of boring everybody who tried to read it. My reason is that there have been so many misinterpretations of what I rather stupidly thought was obvious." The most glaring example, if that is the right phrase, relates to the statement in my written evidence to the Select Committee that "I regard the fundamental arguments in favour of the customer-contractor principle as self-evident", or the first sentence in paragraph 6 of my part of the green paper "This report is based on the principle that applied R and D, that is R and D with a practical application as its objective, must be done on a customer-contractor basis". The justification of this statement, which I still believe to be self-evidently true, would have required a considerable but in my view unnecessary expansion of my report. As, however, my view may be wrong, the justification for it is as follows:

- (1) Applied R and D is done by somebody. I call that somebody the contractor, but the contractor could equally well have been called the scientist, the engineer, the mathematician, the research worker, the boffin, or any other word or phrase used to describe or identify that somebody who does the R and D.
- (2) Applied R and D is an activity with a potential or actual application. Otherwise the adjective "applied" would not be used.
- (3) An application is a use which in turn requires that there is a user, who could equally well be called a customer*, a representative of a customer or user, or even a customer or user surrogate.

I submit that (1), (2) and (3) justify my claim that the sentences in question are self-evidently true. But is it really necessary to enunciate these platitudes? Apparently yes (according to the critics). So I am sorry I did not do so before.

*While usually (and ideally) the customer commissions applied R and D and pays for it, one can easily envisage cases in which the objective which requires R and D was achieved before a customer had paid for it. As an example, a research establishment might approach a potential customer, saying "We have discovered an economic replacement for the additive lead tetraethyl in petrol. We would like to sell it to you at a price which will include the cost of the R and D we did to achieve this objective."

working on the report there was no talk of transferring funds to other departments.

Sir Frederick's discussions with Lord Rothschild during the time when Rothschild was preparing his own report were limited to one formal session, although there were also several informal discussions. Sir Frederick admitted, however, that chapter 3 of Lord Rothschild's report—the chapter that deals with the research councils—did not take any account of what he had said. In spite of this, Sir Frederick felt that the CSP report and that of Lord Rothschild were “more than compatible”. Sir Frederick amplified this point of view by saying that if the customer-contractor principle is accepted, then there would be a need to “identify the work to which it should be applied”. In order to do this a body would be needed to “coordinate and deal with the Rothschild proposals”. Such a body is evidently to be identified with the Board of the Research Councils proposed in the Dainton report.

The CSP report “stood in its own right” according to Sir Frederick, but it had been overshadowed by the simultaneous appearance of Lord Rothschild's report. Sir Frederick regretted the dearth of press comment on his report and he said that he would even now like to see greater discussion of his proposals. Sir Frederick also said that if the Rothschild proposals were implemented then the CSP proposals had to follow suit. In fact the motto should be, according to Sir Frederick, “if Rothschild then Dainton”.

METRICATION

Scarcely to the Point

THE government's long awaited white paper on metrication, published this week, is not so much a policy as an abdication of responsibility.

Although the white paper is peppered with statements on the necessity of Britain going metric—there are “compelling economic reasons” for the change, and “the time has now come . . . to ensure the orderly completion” of metrication—the government plans to do little to ensure that metrication takes place. There will be no M-day, and although the general target for industry to complete metrication remains 1975, it will not matter if the transition stretches into 1976 or 1977.

Speaking at a press conference on Monday, Mr Nicholas Ridley, Parliamentary Under-Secretary of State for Industry, said that the speed of the change “is not being dictated in any way by the Common Market”. The EEC wants exclusive use of prescribed metric units by January 1, 1978, but there is provision for Britain to continue to use certain imperial units until December

31, 1979, and it will be possible to extend the use of imperial units even after that date.

The core of the government's policy is as follows:

- Industry will continue to change at its own pace. The fact that 80 per cent of Britain's exports go to countries which already use the metric system, or are due to shortly, is seen as an incentive to industry to complete the change. The government, however, will not attempt to hasten the changeover by insisting on the use of metric standards in its own purchasing.

- The government will ensure that those goods sold in standard imperial packages under the Weights and Measures Act—butter, tea and sugar, for example—will be marked in metric as well as imperial measures. After consultation with industry and consumers the government proposes to allow these goods to be sold in round metric measures. As a result it is expected that imperial units will gradually fall out of use.

- The government intends to allow beer and spirits to be sold in metric or imperial quantities, although it is felt that to avoid confusion both should not be available in the same bar.

- Road signs are “unlikely to be changed for a long time to come”.

- The government declares that the use of the metric system in schools is a matter for local education authorities and teachers—no pressure will be applied by the government to enforce a change.

- The Metrication Board, which has been subjected to a government review, will continue to function as at present.

First reactions to the white paper are mixed. The Confederation of British Industry (CBI) and the British Standards Institution (BSI) both welcome the

document as an end to a long period of uncertainty about the government's attitude, but the BSI condemns the white paper for its “lack of decision” and “failure to set a programme”. The Metrication Board is cautiously enthusiastic, declaring that the “impetus to change to metrication should be ensured” by the publication of the paper, but a spokesman for the National Union of Teachers said that although the union was much in favour of metrication, it was “bitterly disappointed” at the failure to provide more direct aid for schools to convert to teaching metric—“a great deal more than pious statements are needed”.

SPACE

Support for CNES

from *la Recherche*

THE announcement at the end of January that Mr Michel Bignier is to be the next director-general of the Centre National d'Etudes Spatiales (CNES) came simultaneously with the French government's reaffirmation of faith in its Diamant B rocket. The government has approved the building of an improved version of Diamant B and so allayed all fears that France is considering the purchase of the more economical American Scout rocket. This has helped make Mr Bignier's position that much stronger.

The future of the Diamant B rocket had been in the balance since December last year when it failed to launch the D2A Polaire scientific satellite. The improved version should be able to lift a payload of 200 kg to an altitude of 300 km in contrast to the 160 kg limit of the present rocket. It is also planned that future launches will have a new second stage based on the launch vehicle Rita instead of the stage that failed last December. According to CNES the first launches with the improved rocket should take place in 1974 and the development cost of achieving this is predicted to be of the order of 100 million francs (£7.7 million).

Although the D2A Polaire satellite did not go into orbit in December, its predecessor launched on April 15, 1971, is still working successfully investigating the distribution of hydrogen in the upper atmosphere. As well as supporting strictly French operations such as the launching of sounding rockets and balloons, CNES in 1971 contributed to several international space programmes. The Eole satellite launched on August 16, 1971, by NASA gathered meteorological information from several hundred balloons in the Earth's atmosphere and CNES also had an experiment during 1971 aboard the Soviet Mars 3 probe.

During 1972 there will be no strictly

Sir Alan Cottrell's Mail

WITH a few days to go before the deadline for comments on the government green paper *A Framework for Government Research and Development*, the Chief Scientific Adviser's office reports that 220 letters have been received. Of these 30 to 40 per cent are comments from individuals with most of the remainder coming from professional bodies and groups of scientists employed at research council institutes or universities. The response from industry has been poor but the research associations have commented as well as some individuals employed in industry.

all-French satellites launched, as the next, and last, of the Diamant B series is not scheduled until 1973. There will still be extensive French space effort in the form of experiments aboard European and Soviet satellites and France will contribute to the European biological experiment, Biostack, that will be on board Apollo 16. There is now some concern that the costly installation at Kourou will not be used to full advantage.

EDUCATION

Question of Quality

HARROW County School for Boys—which numbers Professor Sir Charles Dodds among its past pupils—is in danger of having its science department decimated. Under proposals made public recently by the London Borough of Harrow—which is committed to comprehensive education—the school will become a high school in 1974, handling children aged between 12 and 16 up to O-level only. This is a change from the original plans, published in January 1971, under which the school would have become a junior college, teaching children between 16 and 18 up to A-level.

The science department of the school feels that the new decision is misguided. It claims to have the best science facilities in the borough—eight laboratories, all equipped to A-level standard, three for physics, three for chemistry and two for biology. The laboratories are equipped with closed circuit television facilities and adequate storage space. Further, the academic record of the school, while not unprecedented, is very sound. Twenty-one boys went to Oxford and Cambridge this year, thirteen of them in science subjects; six of these won open awards. Over the past ten years 70 per cent of those taking A-level in science and mathematics have been successful, and a high percentage—up to 80 per cent in some subjects—have gone on to science degree courses. Further, the science department produces an annual science journal, *Enquiry*, under the editorship of a senior boy, which circulates to twenty-five countries around the world. With such a record the school feels it should become a junior college—as under the original proposals—rather than a high school.

The new role—as a high school teaching to O-level only—arises because the Secretary of State for Education has indicated that the borough's first co-educational plan would be rejected unless some provision was made for single sex education. The Roman Catholic schools in the area also said that they could not fall in with a co-educational plan. Alderman Grange, chairman of the education committee,

has stated that to meet this requirement Harrow County School was put into the high school list as a school for boys only, and Whitefriars, a mixed secondary school that was previously to have become a high school, was transferred to the junior college list.

Whitefriars, however, has, at best, only four general science laboratories of O-level standard—half the quantity and below the quality of Harrow County School's laboratories. Mr N. Sampson, deputy-director of Harrow Education Department, said last week that “no doubt some of the science equipment will be moved to Whitefriars from Harrow County and in due time Whitefriars will be extended”.

But precise details of what will happen to the staff, the equipment and the children of the schools will not be worked out until the scheme has been approved in principle; however, the uprooting of laboratory equipment and possible dispersal of a staff that has proved its worth does seem pointless if there is a viable alternative to the present scheme, and Circular 10/70 from the Department of Education and Science, on the reorganization of secondary education, does state that the “wise use of resources” is to be one of the chief principles determining reorganization.

So far tempers in Harrow have remained cool. The school has as yet no complaint about lack of consultation—that is taking place over the next few weeks—but it is concerned that a decision on the school's future may be taken without a proper realization of the nature of the school. A high school for boys does have to be found from somewhere, but with seventeen schools involved in the reorganization there must be alternatives. What really concerns Mr J. Avery, headmaster of Harrow County School, is that if the proposals go through they will “smash by dispersal a highly trained staff and some sophisticated equipment”. Certainly if the plan is approved, science could be the loser in the area.

SCIENCE POLICY

Time to Agree

SIR BRIAN FLOWERS, chairman of the Science Research Council, speaking at the University of Manchester last week urged British scientists to stop being emotional about Lord Rothschild's proposals for the reorganization of government research and development and “to concentrate all efforts and attention on influencing the drafting of the white paper due to be published in April”.

Sir Brian, a staunch opponent of Lord Rothschild's scheme for applying the customer-contractor principle on a large scale to the research councils, said that the green paper was now irrelevant

and that it must be realized that the problems are difficult and cannot be solved by a simple administrative formula. The solution, according to Sir Brian, has to be an evolutionary one founded on “in depth studies of various fields of science”.

One of the problems to be solved, according to Sir Brian, is that of optimizing the match between scientific capabilities and national objectives. The research councils bear the brunt of this matching process which becomes most acute in the difficult area of strategic research. According to Sir Brian, it is in this area that the application of the customer-contractor principle breaks down because there is likely to be more than one department needing to use the results of the research. To deal with such a situation Sir Brian would like to see a radical decentralization carried out in government departments so that consultation at all levels through committees and councils can bring together interested parties.

Sir Brian said that one implication of the decentralization would be the setting up of the Board of the Research Councils as recommended by Sir Frederick Dainton so that the efficiency of the programmes of the research councils could be increased.

Sir Brian stressed that it was important for the scientific community to impress upon the government that they were serious about their objections to the Rothschild proposals. A great danger was that the government would implement the Rothschild proposals because they appeared simple and were couched in terms that politicians could understand. A stumbling block for the scientists was that accountability in science was something that non-scientists had difficulty in understanding and it was possible that decisions would be made without a full understanding of the problems.

ENVIRONMENT

Pollution in Ireland

from a Correspondent

IRELAND has so far escaped the worst ravages of pollution, but there is no room for complacency about the future. This was the introductory theme of Mr Robert Molloy, Irish Minister for Local Government and spokesman on pollution, when he opened a public meeting in Dublin last week that was a prerunner of the United Nations Conference on the Environment to be held in Stockholm in July. He announced that a government inter-departmental working group on air and water pollution in the 26 counties would soon present a report with recommendations for legislation.

The attention to be paid at the

Stockholm conference to the problem of pollution in underdeveloped countries has underlined the importance of a recent publication by the Institute of Chemistry of Ireland. The booklet presents the first publicly available body of evidence on industrial pollution in Eire. The voluntary survey was conducted by a committee chaired by Professor D. C. Pepper of Trinity College, Dublin, who emphasized in the report that "there already exists in Ireland a significant pollution problem that is difficult for industry to control, and that action on many fronts is needed if the problem is not to get rapidly worse".

The survey was made in conjunction with the Confederation of Irish Industry which sent a questionnaire to all its members. More than a third of the 385 firms that responded admitted polluting water to some degree, with a half of these discharging raw sewage (a few reporting effluent with highly toxic constituents, for example, cyanide salts, cadmium, and chromium); a fifth of the firms reported some degree of air pollution and nuisance from excess noise, while a few firms also reported difficulty in obtaining adequate sewerage and solid waste disposal, especially in rural areas.

The need to inform Irish firms about pollution abatement was revealed by the survey that showed that 75 per cent of the firms spent nothing on pollution control in 1971. More than 50 per cent of the firms claimed that their effluent disposal was not subject to local authority regulations—a situa-

tion that the report considered to be very unsatisfactory.

The consensus of opinion among the firms was that an injection of public money would provide a strong incentive to cure the problem of industrial pollution—the larger firms preferring special tax relief, whereas the smaller ones wanted outright cash grants. Most of the firms who were able to estimate the cost of their pollution problem considered that production costs would increase by 3 per cent as a result. This trend in financial thinking was seconded at the meeting by Mr Molloy, who felt it was a public responsibility to clean up pollution. These ideas, however, are at odds with the notion current in some environmentalist circles that the "polluter must pay".

PALAEOLITHIC

Mammoths in the Arctic

from our Soviet Correspondent

PALAEOLITHIC remains have been discovered further north than any previously found, according to reports from the Yakutsk Laboratory of Archaeology of the Siberian Branch of the Soviet Academy of Sciences. While investigating the famous "graveyard of mammoths" in the Berelekh region during October 1971, an archaeological team under Professor Yu. Mochanov discovered a number of stone tools as well as some nephrite ornaments.

No description of the artefacts has been given so far; but their importance, it would seem, lies primarily in establishing the limit of palaeolithic settlement into the Arctic. Although Yakutia has long been known as a centre for mammoth ivory—there was a flourishing market in it until the end of the nineteenth century—there has been no evidence until now of palaeolithic mammoth hunters in the area. Professor Mochanov would attribute the "graveyard" to the activities of such hunters—in which case two very interesting problems arise. The first is the long-standing puzzle of how palaeolithic man killed his mammoth, since no suitable weapon has yet been found (pit-falls or driving the beasts over a natural precipice seem the most probable answers). The second, perhaps more important puzzle is provided by the ages and condition of the Berelekh mammoths at the time of death.

At the Predmost site in Czechoslovakia a significant proportion of the bones discovered belonged to half-grown and immature mammoths, and it has been suggested that these were the preferred target of the hunters and that the taking of a fully mature beast was a rare and notable achievement; in which case, this constant thinning out of the

new generation, together with the long gestation time of all elephants, may have contributed to the extinction of the mammoth.

So far, Professor Mochanov has revealed nothing that throws light on these intriguing problems. He seems more interested in establishing the northernmost limits of palaeolithic man, and to this end is planning an expedition for this spring to the Novosibirskie Islands of the Arctic ocean, to seek a possible further outpost of palaeolithic occupation.

INNOVATION

Venture à la Française

A NEW company called the Société d'Etudes pour le Financement de l'Innovation, or SEFINNOVA, has been set up in France. Such a venture follows directly from the *White Paper on Innovation* (see *Nature*, **234**, 166; 1971) that was published by the French government in October 1971. One of the aims of this publication was to create financial corporations to spur innovative processes and SEFINNOVA is the first result of this aim.

The principal author of the white paper was Mr Christian Marbach who was at that time deputy director of research and programmes at the Ministry of Industrial and Scientific Development and it is the same Mr Marbach who will head the new company. The seven members of the staff of SEFINNOVA and its 2.5 million franc capital are at present more modest than the company's ambitions. Intended as a trial effort, SEFINNOVA will, if successful, drop its E for Etudes and be redesignated SOFINNOVA, especially if it can raise considerably more capital than it has at present.

The company has an interministerial watchdog committee and its principal stockholder is the state owned Crédit National with some private firms also holding shares. Thus the company will be free to pick the brains of public organizations in its search for new ideas and it should prove to be a powerful instrument of the French government's strategy.

Supporters of ventures in the research and development field usually support the man rather than the idea. SEFINNOVA is going to break with this tradition by making its money available to unsponsored ideas, those that result from "Here's my discovery, you do something about it." The Paris based firm will then seek out suitable entrepreneurs to develop the idea and make it marketable. Whether SEFINNOVA will sink or swim will depend on whether it can be selective enough in the choosing of winning designs, processes, products and services.

Mathematical History

THE concern of some British mathematicians with the development of the history of mathematics as a subject has led to the formation of the British Society for the History of Mathematics. The society's first president is Dr G. J. Whitrow, Imperial College, University of London, and now, eight weeks after its formation, the society boasts 80 members. These members, according to Dr J. M. Dubbey of Thames Polytechnic, secretary of the society, are evenly distributed between universities on the one hand and polytechnics and colleges of education on the other. The society aims to encourage research in the history of mathematics as well as to support the teaching of the subject. It is planned to achieve these aims by holding regular meetings and conferences but there is no immediate plan to produce a journal of the society.

NEW WORLD

Drug Addiction

from our Washington Correspondent

FOR the past seven months, the Special Action Office for Drug Abuse Prevention, a key element in President Nixon's much publicized war against drug abuse, has been uncertain of its future. Set up in June, 1971, the office has been waiting for the Congressional mill to grind out legislation that will not only formally recognize the office as part of the executive, but which will also set down the terms under which it will operate. But at last it seems that Congress is nearing the end of its deliberations, for both chambers have now unanimously passed bills that will give the office legal existence, and, although many important aspects of the office's operations are yet to be decided, it is already clear that Congress is not willing to hand over to the director of the Special Action Office the full powers that President Nixon originally asked for.

What is also clear is that public concern about drug addiction and political pressures for quick and easy solutions to the problem will make considerably more money available for research in all fields connected with drugtaking. The actual sums of money likely to be voted by Congress, and how they are to be spent, must await the results of a conference committee which will soon meet to sort out differences between the bills passed by the Senate and the House of Representatives.

When the conference committee does meet to strike a compromise on the bills, it will have a difficult task on its hands. On the one side there is a \$1,700 million extravaganza voted by the Senate for a five-year programme weighted heavily towards helping the states to combat drug addiction within their own borders, while on the other there is the more modest programme agreed on last week by the House of Representatives, which asks for \$421 million to be spent over three years on new programmes to be coordinated by the Special Action Office. What is chiefly at stake is the extent of the programmes over which the Special Action Office will have jurisdiction; less in doubt is how the office will exercise its power, for the bills are in relative harmony on that aspect.

When Mr Nixon first sent the legislation to Congress to set up the Special Action Office, he asked that the director be given such extraordinary powers that Congress could hardly fail to curb them. What was suggested was

that the director of the office should be able to control the budgets of each agency concerned with rehabilitation of drug addicts and with research and education on drug addiction, even to the extent of withholding money appropriated by Congress and transferring funds from one agency to another. The bills passed by Congress have, however, divested the director of the power to transfer money and he will also be unable to operate programmes directly from the White House. But he would still be left with a sizeable stick with which to beat federal drug programmes into a co-ordinated approach to rehabilitation and education.

Both bills would give the director of the Special Action Office the same power over drug abuse rehabilitation and education programmes as the Office of Management and Budget now wields. But his actions would be more open to Congressional monitoring than those of the Office of Management and Budget, for the House bill, at least, specifically required that a report be submitted to Congress each year, and that the director be available for questioning by committees.

But even those slightly blunted powers can be expected to raise some departmental jealousies, for established administrators of drug rehabilitation programmes will naturally take unkindly to the type of control that the director of the Special Action Office can wield from the White House. The jealousy was perhaps best phrased by Olin E. Teague, a Representative from Texas, during the debate on the House bill last week. Teague, who is chairman of the House Committee on Veterans' Affairs, tried to keep the Veterans' Administration from being organized by the Special Action Office by offering an amendment designed to keep the drug programmes of the VA under the control of the Office of Management and Budget. In a three-sentence opening speech on the amendment, Teague told his colleagues all this amendment does "is to prevent Dr Jaffe (Nixon's nominee as director of the Special Action Office) from being the dictator over the Veterans' Administration's drug program". The amendment failed by the close margin of 174 to 196.

Apart from agreement over the fact that the Special Action Office should be established, and how it should exercise its powers, however, there is little in common between the bills passed by the House and by the Senate. The

House bill, for example, seeks to appoint a 15-member National Advisory Council for Drug Abuse Prevention, to advise the director of the Special Action Office on his functions and to recommend programmes which it feels could usefully be adopted by the federal government.

The Senate bill, on the other hand, seeks to establish a Strategy Council, charged with the responsibility of drawing up longer term plans for federal efforts designed to curb drug abuse, and with evaluating present drug programmes.

What the House bill does is essentially to set up the Special Action Office in the White House and give it the power to direct and control existing drug abuse programmes that are not related to law enforcement, while the Senate bill seeks to add on to that basis a number of new programmes and initiatives. Nearly all the additions are the work of Senator Edward M. Kennedy's Health Subcommittee of the Senate Committee on Labor and Public Welfare, and they open the way for another conference committee tussle between Kennedy's subcommittee members and members of the Public Health and Environment Subcommittee chaired by Paul G. Rogers, chief architect of the bill passed last week by the House of Representatives.

The additions to the Senate bill include the setting up of a National Institute on Drug Abuse within the National Institute of Mental Health, to draw together the work of the Department of Health, Education and Welfare in the field of drug abuse, the establishment of a programme for giving grants to help states to set up their own drug abuse treatment and prevention plans, and a \$1,300 million plan to give project grants for all sorts of activities in the drug abuse field.

While the outcome of the conference committee's deliberations is difficult to predict, because the two bills are so different in scope, it is probably safe to say that whatever happens at the conference table, the funding authorized for research into drug addiction, including projects aimed at finding an effective agent to block the craving for a drug, will be considerably increased. The budget request for the 1973 fiscal year submitted by President Nixon to Congress recently calls for expenditure of \$49 million on narcotic research, while the House bill calls for an extra \$20 million on top of that; the Senate plan also contains provision for considerable funding.

SCIENCE POLICY

Rothschild's Recipe in the United States

Professor Harvey Brooks, Dean of the School of Engineering at Harvard University and Chairman of the Committee on Science and Public Policy, National Academy of Sciences, analyses the Rothschild and Dainton reports.

SCIENCE policy watchers have noted a tendency towards convergence of the organizations for management of government research and development in the United States and Britain (*Science, Growth and Society, a New Perspective*, 66-69, OECD, 1971). The present British Government green paper, however, underlines for me, an American observer, the large gulf that still exists between the two systems. I find myself struck by the mildness of Lord Rothschild's proposals when compared with American reality. The American system has, of course, operated from a formal standpoint almost entirely on the "customer-contractor" principle since the end of the Second World War, although the reality has not been as rigid as the legal form.

If one were to equate the National Science Foundation of the United States with the Science Research Council in Britain, then the NSF accounted for only 2.5 per cent of the US Government research and development budget in the 1972 financial year and less than 3 per cent of the President's new budget for 1973. By contrast, the corresponding figure for the SRC is 7.9 per cent. If defence research and development is excluded in both cases then the comparable figures for 1972 are 5.15 per cent for the NSF and 13.33 per cent for the SRC. The figure is projected to rise to 5.7 per cent for the NSF in consideration of the expansion of RANN and other applied programmes in 1973. In fact, it would probably be fairer to compare the total DES budget with that of NSF. In this case the DES fraction rises to 28.4 per cent of the civil science budget, and would be reduced only to 21.2 per cent were the Rothschild recommendations to be fully implemented. Thus even after Rothschild the percentage of civil science in Britain which is formally independent of the customer-contractor principle would be nearly four times greater than in the USA.

Some, of course, would quarrel with the above comparison. The National Institutes of Health, for example, be-

haved in the past in practice more like a research council than like a regular ministerial department. Through a special relationship with the Congress, they were able to act quite independently with respect to their parent agency, the Department of Health, Education and Welfare, and this was important because they accounted for more than 45 per cent of the support of academic research. Although this independence was realized in practice, however, it did not exist in form, and present trends seem to foreshadow a rapid transition towards the substance as well as the form of the customer-contractor principle in this agency.

Since we in the United States did live fairly comfortably with the customer-contractor principle until recently, and American science, on the whole, prospered under it, I find it rather difficult to empathize with the cries of anguish which the Rothschild report has evidently elicited from the British scientific community. On the other hand, I am also struck by Lord Rothschild's apparent obliviousness to some of the evident weaknesses and dangers of the American system, which he seems to admire without attribution. The instability in funding, the effective lack of concern with the integrity and viability of scientific institutions—especially the universities—the wasteful competition for control over glamorous or spectacular technical programmes, the confusion of technological virtuosity with scientific achievement, the increasing obsession with narrowly conceived "social relevance", sometimes to the detriment of scientific quality, the exacerbation of competitiveness, "grantsmanship", and political manoeuvring in the scientific community—all of these negative symptoms, which are increasingly appearing in the American system, are ignored in the Rothschild report. It may be, with the generally greater orderliness and decorum in British politics and British society generally, that these symptoms will not appear in Britain, but the possibility does deserve comment.

The Rothschild report attacks the distinction between "tactical" and "strategic" science made by the Dainton report, and also criticizes its stress on the difficulty of distinguishing between basic (or "pure") and applied science. I feel the argument is largely a semantic one, but I also think that Dainton is right in stressing the existence of a category of research which cannot be comfortably subsumed under the customer-contractor concept. I take it that tactical research is research whose detailed goals are specified in advance largely by the customer. Strategic research, on the other hand, is research directed at, or at least influenced by, practical goals, but ones which the customer does not yet know he wants. The US Office of Scientific Research and Development during the Second World War was organized to do military research and development under civilian leadership, and specifically had the power to initiate research or development without a "requirement" from the customer. After Sputnik it was found necessary to create the Advanced Research Projects Agency somewhat in the image of OSRD as a separate civilian "fourth service" reporting directly to the Secretary of Defense with the power to initiate developments without a military requirement from one of the services.

It seems doubtful that weather satellites would ever have been developed by the US Weather Bureau, or nuclear reactors by the Federal Power Commission, or radioactive tracers by the Department of Health. There is a stage between basic research and application which must be filled by science-generated rather than customer-generated research effort and yet which is not pure research. Eventually such research has to be marketed, that is, sold to a customer, but substantial scientific resources must be invested in the absence of a definite customer before this can be done. Most large private companies recognize this principle in their corporate research laboratories, which are separate from their product or functional divisions and report at the highest corporate level.

Lord Rothschild apparently hopes to provide for this need through a general research surcharge on applied research and development in the departmental research establishments, estimating that this will amount to about £54 million (paragraphs 15 and 18). A somewhat analogous system has been tried in the

United States in several forms. Industrial contractors with the Defense Department and NASA are permitted to include an item for "Independent Research and Development" as a portion of overhead charges on procurements for government end-use. The content of the work is subject to varying degrees of supervision and review by scientific teams from the procuring agencies, but the company has proprietary rights in the results. Government laboratories of the Defense Department are also frequently permitted a percentage of their total budget for independent or locally initiated work. The most frequently cited example of the benefits of such self-generated work is the development of the Sidewinder missile initiated at the Naval Ordnance Test Station in Pasadena. This was carried out over the objections of the customer (the US Navy Bureau of Ordnance), and it was only "sold" after endorsement by a high level DOD advisory group chaired by Dr Donald Hornig, later to become Presidential Science Adviser. The re-

supplier rather than being dictated unilaterally by the client. When the sponsor tries to override the scientific judgment of the contractor, success is unlikely. There is a danger that Lord Rothschild's prescription may be interpreted as a relationship between a boss and an underling rather than a true collaboration between user and performer. If either the customer or the contractor has no viable alternatives, the one to go elsewhere for help and the other to go elsewhere for sponsorship, the relation is likely to fail. How this delicate balance of bargaining power works out in practice depends on people and attitudes as much as on organizational forms, a point which the Rothschild report seems to recognize (paragraph 21). The recommendation that a research council is not to be permitted to reject a proffered contract without agreed reasons, assented to by the sponsor, would in my view tip the balance too far in favour of the customer, to the ultimate disadvantage of both customer and performer.

especially at the hands of the powerful Congressional committees that oversee the parent agency. It will be very valuable if Britain can successfully develop and implement this concept of the broad-spectrum multi-purpose laboratory, with which many ministries and the private sector may contract for technical work.

Some of Lord Rothschild's recommendations regarding scientists in the civil service also represent a move towards the American system, in which the formal distinction between scientists and administrators has always been non-existent, and where administrative training programmes for scientists are well developed. More than 50 per cent of the top two grades of the US civil service are occupied by technically trained people, including social scientists. This is probably a necessary precondition for the satisfactory operation of the customer-contractor principle, a fact that is perhaps not sufficiently emphasized by Lord Rothschild. Although I agree that non-scientists occasionally make good laboratory directors or heads of technical agencies, there is a credibility gap between technical and non-technical people which makes this a rarity, and I feel that Lord Rothschild is misleading in this regard (paragraph 45). Even if such administrators were formally competent it would be difficult for them to earn the confidence of the technical people with whom they had to deal, especially if hard and unpopular decisions were necessary.

Another minor comment concerns Lord Rothschild's somewhat capricious use of the word "long term" as synonymous with "taking a long time" rather than its more common meaning of relevance to goals that lie relatively far in the future. The customer can seldom project his specific needs for knowledge more than a few years into the future. Long experience suggests that for such unforeseeable needs the most economical and efficient procedure is a research strategy guided by the conceptual structure of science, that is, the requirements for understanding and generalization. This consists of a mixture of "pure science" and "strategic research", if I may be permitted to mix Lord Rothschild's and Professor Dainton's terminologies. To characterize the search for conceptual understanding as "scientific roulette" (paragraph 6) seems somewhat of a caricature, though it is recognized that practical research goals should be defined when the state of scientific knowledge makes this realistic and not a mere exercise in semantics.

On balance, though occasionally blemished by overstatement, Lord Rothschild's proposals seem sensible and well argued and, certainly from the point of view of an American, hardly very radical.

Table 1 Lord Rothschild's Proposed Reallocation of Research Council Budgets

Council	Present budget (£ million)	Transferred to department(s) (£ million)
SRC	50.9	0
SSRC	2.2	0
ARC	18.7	14.5
MRC	22.4	5.6
NERC	15.3	7.6
	£109.5 million	£27.7 million

search and development budget of the Defense Department also includes an item for "military sciences" which amounts to about 8 per cent of the total and is not programmed against any specific enduse. About half of this is spent in academic institutions, with emphasis on locally generated and managed programmes such as the 25-year-old Joint Services Electronics Program. Academic research supported by all mission-oriented agencies constituted about 9 per cent of all mission-oriented (that is, departmentally supported) research and development of the US Government in the 1972 financial year. Thus, however interpreted in terms of US experience, Lord Rothschild's surcharge seems to reasonably consistent.

Much depends on how the customer-contractor relationship is interpreted in practice. When the customer is reasonably sophisticated about the research and development process and is represented by competent scientists, the concept has been successful in the United States. It succeeds best when the relationship is a negotiated one between client and

The Dainton report took strong exception to moving the nominally mission-oriented research councils into cognizant ministries. The chief objection was that a single council usually serves the purposes of several ministries and even the private sector (paragraph 34). This point was ingeniously met by Lord Rothschild's customer-contractor principle, which makes it possible for a single research council to serve several users simultaneously through contracting. There is still the danger that everybody's business will become nobody's business, especially in times of budgetary stringency. This has certainly been one of the lessons learned in the US during the past five years of budgetary competition from social programmes. This contingency would presumably be met in Britain out of the council's directly appropriated budget.

The idea of multi-purpose applied laboratories within government has been poorly developed in the United States. The so-called "national laboratories" are largely the captive of single agencies, and offer their services to broader national missions at considerable political risk,

Proposal for a Constructive Response

Dr G. J. Leigh, School of Molecular Sciences, University of Sussex, outlines a possible alternative to the Rothschild proposals.

It seems clear, whatever scientists think of Lord Rothschild's proposals, that the research council system will be altered. The government has accepted and endorsed the customer-contractor principle, and has invited comment, not on whether it should be put into effect, but on how it should be implemented. It would be unwise to hope that the outcry of a few thousand tormented souls will deter the government from doing what it believes to be best. Discussions which simply emphasize shortcomings of the Rothschild report are therefore beside the point and not constructive.

There are no accepted concrete criteria by which one can judge the effectiveness of forms of organization for promoting research and development. Rothschild's main objection to the present system would appear to be that a great deal of the applied research undertaken by the research councils is not commissioned by a customer. This, he says, is wrong. There is, however, persuasive evidence that this research is nevertheless used, and used successfully. For example, the value of the gross output of food produced in this country is greater than that produced in Canada, Australia or New Zealand, and it would be surprising if the Agricultural Research Service were not, in part, responsible. A more trenchant criticism of the present organization would therefore have been useful.

Likewise, Rothschild presents no proof that the customer-contractor relationship promotes greater effectiveness. There must, however, be information obtainable on how the principle works in other circumstances. Industry must cost its research and development budget closely, and Lord Rothschild has had close experience of at least one large company. The research associations and scientific consultants must work generally on a customer-contractor basis. Certain other countries arrange research programmes centrally, and it would have been instructive to have had a critique of research and development in, say, East Germany or the Soviet Union.

In spite of the lack of evidence for the value of the proposed upheaval, the government wishes to alter radically the present organization. The proposed changes will, for the scientist, introduce

stress and insecurity. The government has not shown itself particularly sensitive to scientists' needs in the past, and this raises general questions about the government's attitude to science, and the whole future of scientists in government research establishments.

Assuming that the changes take place as suggested by Rothschild, it would be valuable for the government to instruct the relevant ministries to draw up a comprehensive list of the research and development problems that they feel should be placed with the research councils. These should then be submitted to the research councils for detailed comment on feasibility, cost and value. Such an exercise would test the abilities of the ministries to formulate programmes, and demonstrate whether the research councils are as inflexible and insensitive to national requirements as their proposed fate would suggest.

The individual research councils should be replaced by a single research council dealing with all the sciences. This would create a large organization, but this does not necessarily promote inefficiency. (The Shell Oil Company seems comparatively effective in spite of its size.) Within the new research council there might well be subdivisions corresponding to the present councils, but it would allow much more flexible arrangements of staff and facilities, both interdisciplinary and between the subdivisions, than is at present possible. Such an amalgamation would avoid some duplication of staff, prevent the submission of the same research proposal to more than one body and, above all, facilitate the formation of multidisciplinary research groups and the dissemination and integration of information between disciplines, a development that the present councils are already encouraging. It would also provide a system more adaptable to future needs than the present one. The new research council would commission its own work in its own establishments and also support work in the universities, as do the councils at present.

The work of the research council would be under the direct supervision of a committee, analogous in some respects to the Council for Scientific Policy, but quite different in that it would not

merely play an advisory role. It would be composed of representatives of scientists, government and industry, and of laymen. It would also possess its own independent scientific staffs. Its functions would be manifold.

It would advise the government on financial, social and strategic aspects of science policy, obtain funds for the research council from the Treasury or the DES and mediate between the sometimes conflicting requirements of scientists and the government. It would go to industry and the ministries and accept advice on the areas of research that would be of the most direct national benefit. It would continuously appraise the work of the research council, and could order it to withdraw support from, or reduce support to, those areas which no longer warranted encouragement. Finally, it could instruct the research council to commission work on particular subjects, for any good reasons, be they social, economic or scientific.

The new committee could also effectively formalize a wide range of presently informal functions. It could attempt to translate the requirements of customers into terms which would satisfy the scientist and show the customer how he might use the scientist's results. A far wider range of customers might then be obtained than Rothschild suggests. It could also consider the balance of pure and applied research, a problem which Rothschild ignores. This will obviously vary with the subject and with time. A particular function could be to encourage the grey areas between pure and applied research. It is difficult to imagine a ministry commissioning work on, say, the linear induction motor before one had been invented. Yet such work can be classified as both pure and applied.

Some such arrangement as outlined would be in the spirit of the customer-contractor principle, in that the potential customer could have work carried out on his behalf if he could convince the committee of its relevance and priority. It would retain for the scientist a necessary degree of autonomy and freedom from direct pressure. It would also allow the democratic process (which Rothschild apparently equates, in my opinion incorrectly, with the customer-contractor mechanism) to work in the control of government science, and to be seen working. It is only by a set of counter-proposals, possibly such as these, that a solution to the mutual benefit of science, scientists and the government can be developed.

NEWS AND VIEWS

A Soft-centred Earth

It has often proved to be the case in geophysics that new methods of observation have quickly led to fresh ideas about the properties of the Earth. The story of the inner core is a current instance, for quite suddenly it seems that questions about the nature of the inner core are being resolved by observations with arrays of seismometers and studies of the free elastic oscillations of the Earth.

Two quite simple arguments show that the Earth has a dense core extending to about half its radius. The moment of inertia of the Earth, estimated from the polar flattening and precession, shows the density to be much greater at the centre than at the surface and the existence of a shadow zone for seismic waves, extending from 100 to 140 degrees from a source, shows that there is a sharp boundary at about half the radius at which the speed of compressional elastic waves suffers a sharp decrease, refracting rays away from the shadow zone. The fall in speed of elastic waves, combined with the increase in density, leads to the conclusion that the core must be liquid, a conclusion supported by the period of nutation of the Earth. The equation of state of the outer part of the core is now understood in some detail and can be compared with that of iron which has been derived from experiments on strong shock waves. The agreement is good enough to suggest that the core is composed largely of iron.

The shadow zone is not, however, completely dark. Some energy would be expected to leak into it through diffraction round the boundary of the core, but more is found than can be accounted for theoretically and Miss I. Lehmann suggested in 1936 that the observations could be explained if the speed of compressional waves increased sharply at a radius of about 1,200 km. Some energy would be reflected at this inner boundary and more would be refracted through the inner core into the shadow zone. For many years, little more could be learnt about the inner core although it was usually supposed to be solid, not liquid, on account of the parallel increase of density and compressional wave speed.

There the inner core lurked in a sort of limbo for some while, but now as a result of new observations it begins to look much more distinct. The important developments in seismology in the past decade or so have been the observation of the elastic oscillations of the Earth as a whole and the use of arrays of seismometers, instead of single instruments, in the detection of seismic pulses. At the 1970 annual meeting of the Seismological Society of America, Engdahl, Flinn and Romney announced that they had used the Large Aperture Seismic Array (LASA) in Montana to detect seismic pulses reflected at a steep angle from the boundary of the inner core. As Bolt and Quamar showed (*Nature*, **228**, 148; 1970), this observation establishes that the inner core boundary is sharp and that the density just inside the inner core must be less than $13,500 \text{ kg m}^{-3}$ (in some models it had exceeded $17,000 \text{ kg m}^{-3}$). The next advance was reported last year

in *Nature* (**234**, 465; 1971) by Dziewonski and Gilbert and discussed in the same issue (p. 438).

The periods of the free elastic oscillations of the Earth can be calculated if the elastic moduli and density are specified as functions of radius, and by comparing the observed periods with those so calculated, the proposed structure of the Earth can be improved. The procedure has been very effective in constructing models of the outer parts of the Earth but does not work so well for the inner core because the amplitudes of the fundamental modes are very small in the inner core and so the mechanical properties of the inner core have little effect on the calculated periods. The amplitudes and effects on the periods are relatively large if overtones can be used, and that is what Dziewonski and Gilbert did. They were able to show that the inner core must indeed be solid and that the speed of shear waves should have the relatively low value of 3.2 km s^{-1} .

Julian, Davies and Sheppard (on page 317 of this issue) have now made a direct observation of seismic signals that travel as shear waves through the inner core. They have used the LASA to find signals that not only arrive at the correct time but also travel across the array at the correct speed, and so they are able to isolate the small signals in the presence of noise. They find that the speed of shear waves in the inner core is about 3 km s^{-1} .

There is now available a rather sharp picture of the inner core. Its radius is about 1,220 km, the boundary is quite sharp, its density just within the boundary is less than $13,500 \text{ kg m}^{-3}$ and the speeds of compressional and shear waves are 10.8 and 3 km s^{-1} respectively. The ratio of shear modulus to bulk modulus is 0.08 , rather less than that for gold or lead in ordinary circumstances—the Earth has a soft centre.

The properties of the core and inner core are so similar that it seems very natural to suppose that the inner core is solidified core material. The rate of change of temperature in the innermost parts of the core is probably rather slow, as is the change of melting temperature with pressure, so that the position of the boundary of the inner core would seem to be quite sensitive to the conditions in the core. No doubt the size of the inner core is very much a consequence of the state of the Earth just now and, although the elastic boundary of the inner core is sharp, it might be that there is some liquid in it—like fudge.

If the inner core is close to the melting temperature, so also is the region of the core just outside it. Convection in the core may then be only just possible. Convective motions are necessary for the core dynamo theory of the Earth's magnetic field and, if they are only just possible, the inherent instability of the dynamo process might be enhanced; the relatively frequent reversals of the Earth's field in the recent past may thus be related to the existence of the inner core.

The size of the inner core is probably peculiar to the

Earth. Indeed, Venus may be the only other planet with a core like that of the Earth and the inference that should be drawn from the present picture of the inner core is that it is very much a matter of the present conditions in Venus whether her core is wholly solid or wholly liquid or in some intermediate state.

It is remarkable, as Bolt and Quamar observed in their communication, that so much can be learnt about a region of the Earth so remote from us, and it is a fine illustration of the power of modern methods in seismology. The new certainty about the inner core now prompts fresh thought about the mechanical, thermal and electrical states of the cores of the terrestrial planets, why the Earth is the only terrestrial planet with a magnetic field and what the course of formation of the Earth may have been to give such apparently special conditions as now exist in the centre of the Earth.—From a Correspondent.

Synthesizing Globin DNA

WITHIN weeks of the announcement in June 1970 of the discovery of reverse transcriptase it had become abundantly clear from repetitious experiments that this enzyme, unlike some other nucleic acid polymerases, is quite promiscuous and will transcribe into DNA synthetic and natural RNAs unrelated to any tumour virus genome. Not a few of the numerous research groups which had begun to work with reverse transcriptase were quick to realize that, quite apart from and irrespective of the part this enzyme may play in the transformation of cells by RNA tumour viruses, this lack of template specificity made reverse transcriptase potentially at least an extremely useful reagent. There seemed to be every reason to hope that it might be possible to use the enzyme to synthesize DNA copies of, for example, mammalian messenger RNAs. And that would of course be tantamount to synthesizing mammalian genes *in vitro*.

All that seemed to be required was an ample supply of the enzyme and some pure preparation of a mammalian messenger RNA which could be used as a template. The first requirement was easily met; the blood of chicks viraemic as a result of infection by avian myeloblastosis virus contains very high titres of this virus from which reverse transcriptase can be purified. A supply of pure mammalian messenger RNA is harder to come by but there was one obvious source, namely the 10S RNA from reticulocytes which is capable of programming the synthesis of globin in cell-free systems which support protein synthesis. At least some of this reticulocyte 10S RNA must be messenger for globin.

Just how many groups during the past year have begun to try to synthesize, with reverse transcriptase, DNA complementary to globin messenger RNA from one species or another remains to be seen, but recent reports are the vanguard of what will no doubt prove to be a not inconsiderable army. In *Nature New Biology* last week Baltimore's group and Spiegelman's group (Verma *et al.* and Kacian *et al.*, **235**, 163 and 167; 1972) reported the synthesis of DNA complementary to rabbit and human 10S globin messenger RNA preparations, and in the January issue of the *Proceedings of the National Academy of Sciences* (**69**, 264; 1972) Ross, Aviv, Scolnick and Leder also report the synthesis of

DNA complementary to rabbit globin messenger RNA.

The experiments of the three groups are essentially identical, although each has added its own small variations on the common theme. Reverse transcriptase, from avian myeloblastosis virus particles, was provided with the four deoxynucleoside triphosphates, suitable concentrations of magnesium ions, purified 10S RNA from reticulocytes and a primer, oligo(dT) chains about a dozen bases long. The primer is added, for, as Baltimore's group have shown, reverse transcriptase initiates the synthesis of DNA by forming a phosphodiester bond between the 3' hydroxy group of a short primer chain, which is hydrogen bonded to the template RNA, and the 5' α -phosphate of the first deoxynucleotide. Fortunately, as Burr and Lingrel (*Nature New Biology*, **233**, 41; 1971) have found, there is a short run of adenylic acid residues at the 3' end of the globin messenger RNAs which presumably provide the oligo(dT) primer chains with the opportunity of hydrogen bonding to the globin messenger RNA template molecules and presenting their 3' hydroxyl groups as a site for initiation of transcription.

When incubated in these conditions reverse transcriptase stimulates the prolonged polymerization of deoxynucleotides into DNA. The reaction depends absolutely on the presence of template and all four deoxynucleoside triphosphates; it is almost totally dependent on the addition of primer and it can be restricted to the synthesis of only single stranded DNA by the addition of actinomycin D. The initial product of this reaction is, of course, a DNA/RNA hybrid molecule, the DNA moiety of which can be isolated by digesting or hydrolysing away the template RNA. The single stranded DNA obtained in this way hybridizes specifically with reticulocyte 10S RNA and it sediments at about 8S. Because the DNA product has a slightly smaller sedimentation coefficient than the template RNA may mean simply that not all the template is transcribed, but alternatively the DNA may be as long as the template RNA but possess a different structure.

The precise length of the transcript DNA, proof that the oligo(dT) primer is actually acting as a primer and occurs at the 5' end of the product DNA, and the role of oligo(dG), which, Verma *et al.* find, can with loss of efficiency of synthesis substitute for oligo(dT), are all points which demand further investigation. But most important of all, it still remains to be proved that the molecules in 10S RNA preparations from reticulocytes which act as templates for reverse transcriptase are the active globin messenger RNA chains rather than some contaminating RNA. Of course, several lines of indirect evidence suggest that that will prove to be the case; the template activity of different preparations of 10S RNA parallels their activity as messenger for the synthesis *in vitro* of globin; of the various RNA fractions that can be isolated from reticulocyte polysomes only the 10S fraction acts as template for reverse transcriptase in the presence of oligo(dT) and so on. None of these findings, however, constitutes rigorous proof that the DNA made *in vitro* is globin DNA.

Assuming, as seems likely, that the single stranded DNA made in this reaction is indeed complementary at least in part to globin messenger RNA and therefore identical to at least the part of the strand of the globin genes from which messenger is transcribed, what can this *in vitro* DNA be used for and why all the excitement?

Simply by feeding into the reaction mixture suitably labelled deoxynucleotides the DNA can be labelled to a very high specific activity; it can then be used as a specific probe either for globin genes or for globin messengers and a host of intriguing questions, which cell and molecular biologists would dearly like to be able to answer, are at a stroke rendered experimentally feasible. It should, for example, be possible to find out how many copies of the globin genes are present in a cell, to find out at precisely what stage in erythropoiesis they are first transcribed and to identify nuclear precursors to cytoplasmic globin messengers and follow their maturation. Moreover, there is every reason to anticipate that other species of mammalian messengers, as they become available, will prove to act as templates for reverse transcriptase and therefore as a substrate for the manufacture of genes, a prospect which in the long run is not without its implications for genetic engineering.—From our Cell Biology Correspondent.

NUCLEAR ENERGY

Salting Away Waste

from our Materials Science Correspondent
 "OUT of this nettle, danger, we pluck this flower, safety." Hotspur's concern was war, but his adage is apt for the nuclear power industry. Because radioactivity poses an unmistakable and ever-present threat to people who work in the industry, safety is taken with extreme seriousness and its safety record is one for other, more fatalistic industries to envy. Nonetheless, "environmentalists" often have their knives into the industry: sometimes they worry about the risk of radioactive release into the air, sometimes about "thermal pollution" but most commonly about the supposed dire consequences of the steady accumulation of highly radioactive wastes.

Undoubtedly there are serious problems here: radioactive constituents are separated by remote-controlled chemical processing from spent nuclear fuel, and the concentrate is then usually melted with appropriate additions to form blocks of chemically very stable glass. This glassy concentrate is so extremely radioactive that, in bulk, it heats itself to a temperature of several hundred degrees C. The glass, sealed in metal containers, is buried in man-made vaults; for the future, as quantities increase, the US Atomic Energy Commission hopes to use disused deep salt mines, because salt deposits are apt to be seismically very stable formations and, as further insurance, being plastically deformable, such formations could resist disturbance by earthquakes. The wastes have to stay put for thousands

of years: the question is, can they be trusted to stay put?

Much theoretical and experimental work has been carried out recently in the United States to assess the safety of salt mines. One curious potential problem arises from the presence in natural salt deposits of small droplets of trapped brine. Because the buried radioactive wastes are literally hot, there will be a temperature gradient in the surrounding salt, and it is known that brine droplets will migrate up a temperature gradient (T. R. Anthony and H. E. Cline, *J. Appl. Phys.*, **42**, 3380; 1971). This could be serious, because the brine droplets could progressively spill out of the cavern wall, increase the humidity in the cavern and hasten corrosion of the sealed containers, and then even the stable glass could gradually be attacked and dispersed into other parts of the mine.

Anthony and Cline, who work at the General Electric R and D Centre in Schenectady, have now pursued this intriguing problem a stage further (*Acta Metallurgica*, **20**, 247; 1972). They predict that when a brine droplet reaches the salt surface, some water evaporates, the brine becomes supersaturated, salt is precipitated and seals off a bubble which now contains both brine and some air or water vapour; and furthermore, that such mixed bubbles will turn round and migrate down the temperature gradient. This they confirmed by direct experiments on a KCl crystal. Typically, a small bubble in a gradient of 3 degrees per centimetre at 40° C migrated a few μm per hour.

Anthony and Cline go thoroughly into the physics of the process and then go on to consider the relevant magnitudes as applied to "nuclear crypts". It seems that in the most unfavourable case, liquid/vapour bubbles could move several metres into the salt in a year, carrying with them any stray radioactive material that might conceivably attach to the cavern wall (possibly because of corrosion by the released water). Beyond the distance of a few metres the temperature gradient caused by the self heating must rapidly attenuate, and Anthony and Cline calculate that, because of trapping at grain boundaries in the salt, there is no risk of bubbles continuing to migrate in the much weaker temperature gradient of the Earth itself, or in its gravitational field. The limited dispersal predicted, however, would complicate later exhumation of the wastes in the unlikely event of this being thought necessary. Problems thus exist even for the strategy of burial in deep salt mines, but they seem to be serious only if the need to exhume totally is regarded as a realistic possibility. What this valuable article illustrates is how much scientific care is devoted to every facet of safety

by those responsible for it as distinct from the cavalier irresponsibility of which they are apt to be accused.

The migration of bubbles in salt is just one example of a whole family of processes of especial relevance to nuclear energy, which includes the migration of bubbles of fission-gas in steep temperature gradients present in ceramic nuclear fuels, the dragging of spherical pores by grain boundaries as they move, and the motion of a dislocation loop in an elastic strain field.

GRAVIPERCEPTION

A Cellular Hypothesis?

from our Plant Physiology Correspondent
 RECENT electron microscopic investigations culminating in the elegant work of Sievers and Dolkman, published in the current issue of *Planta* (**102**, 160; 1972), look set to resolve and place on a rational footing one of the oldest controversies in plant physiology. The mechanism by which plant roots perceive the force of gravity, and translate the stimulus into the directional growth response known as geotropism, has exercised the minds of botanists for more than a century. As long ago as 1886, Berthold suggested that gravity was perceived by the movement through the root cells of special starch containing plastids known by analogy with the labyrinthine receptors of animals as statoliths. This theory was amply supported by the classical observations of Haberlandt and Nemeć who, seventy years ago, demonstrated an almost perfect correlation between the occurrence of movable statoliths and the existence of a geotropic response. The 1920s, however, saw the rise of the all-conquering auxin and the development of the Cholodny-Went hypothesis in which it was postulated that unilateral exposure to a gravitational stimulus led to a redistribution of auxin towards the lower side of the organ. Because root growth was inhibited by high levels of auxin such a redistribution would cause the observed downward geotropic curvature.

Although the Cholodny-Went hypothesis was restricted to auxin movements and did not attempt to explain the perception of gravity, nevertheless the statolith theory became virtually discarded. Later, the picture became more complicated by the work of Brauner and his colleagues, who observed a change in electrical potential across the root upon turning it horizontal. Brauner claimed that this so-called geo-electric effect was part of the perception mechanism and caused the redistribution of auxin by an electrophoretic migration. There is, however, no known physical phenomenon which could account for the geo-electric effect,

and it is probably a result, rather than the cause, of the geotropic growth changes.

Fortunately, the nineteen-sixties have seen a revival of interest in the mechanism of geotropism, led by the review of Audus (*Symp. Soc. Exp. Biol.*, **16**, 197; 1962) in which he pointed out the previously ignored fact that the perception of gravity must involve the gravity-mediated movement of some part of the perceptive cell. Calculations showed the smallest possible contender to be the mitochondrion and the most likely a form of plastid, heavy with starch, known as an amyloplast. Following up this concept, Griffiths and Audus (*New Phytol.*, **63**, 319; 1964) used the electron microscope to show that the gravi-perceptive cells of bean roots contained large, movable amyloplasts with all the properties of statoliths. Juniper and French (*Planta*, **95**, 314; 1970) then observed that the contents of the perceptive cells of the maize root cap were organized in a special way; in the morphologically lower part of the cell there was often a distinct concentration of endoplasmic reticulum above which rested conspicuous amyloplasts and it seemed likely that the movement of the amyloplasts under the action of gravity would affect the endoplasmic reticulum in some way. The idea thus developed that the relationship between the amyloplasts and the endoplasmic reticulum was important for graviperception.

The latest electron micrographic studies of Sievers and Dolkman provide a strong indication of the nature of this relationship. Using cress roots they have shown that during the ontogeny of the perceptive cells of the cap, a complex of rough endoplasmic reticulum develops in all cases just above the lower transverse wall. The central cells of the perceptive region are cylindrical and their longitudinal axes lie parallel to the longitudinal axis of the root. In these cells, the cisternae of the endoplasmic reticulum are oriented parallel to the lower transverse wall and a small number of large amyloplasts lie directly above them. The peripheral cells of the perceptive region, on the other hand, have their long axes oriented diagonally across the root axis with the cisternae of the endoplasmic reticulum lying in the angle between the lower transverse wall and the outer longitudinal wall, again with amyloplasts directly above. When the root is moved to a horizontal position the amyloplasts in the cells on the now upper side of the root move away from the endoplasmic reticulum complex, whereas in the cells on the side which is now lower they move closer to the reticulum complex. This process serves to set up a lateral polarity across the root cap, in which the degree of contact between the amyloplasts and the

endoplasmic reticulum increases from the cells on the upper side of the perceptive region to those on the lower side. It is not inconceivable that the degree of contact between the amyloplasts and the endoplasmic reticulum could in some way regulate the metabolic activity of the reticulum leading to a metabolic gradient across the root cap. The asymmetric formation of growth regulatory substances such as the inhibitor found by Gibbons and Wilkins in corn root caps (*Nature*, **226**, 588; 1970) could then be readily understood.

This is not a new hypothesis because it was first enunciated by Audus in 1962, and can be traced back to the light microscope observations of Nemeč in 1901, but the clear-cut results of Sievers and Dolkman present the best argument yet for accepting it.

PLANT VIRUSES

Better Rhubarb?

by our Botany Correspondent

THERE are signs that the elimination of viruses from specially cultivated stocks could lead to increased yields of rhubarb. The first indication that larger crops might be produced comes from the National Vegetable Research Station near Warwick, where Drs D. G. A. Walkey and V. C. Cooper have found that a greater weight of rhubarb can be grown from plants which are not infected with viruses (*J. Hort. Sci.*, **47**, 37; 1972).

As with many other valuable fruits and vegetables, the British commercial cultivars of rhubarb (*Rheum rhaponticum*) have been found to be widely infected with viruses, including turnip mosaic virus, cherry leaf roll virus and

Electron Microscopy and Metal Chalcogenides

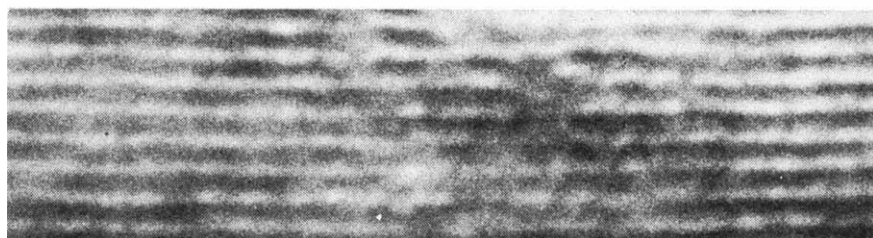
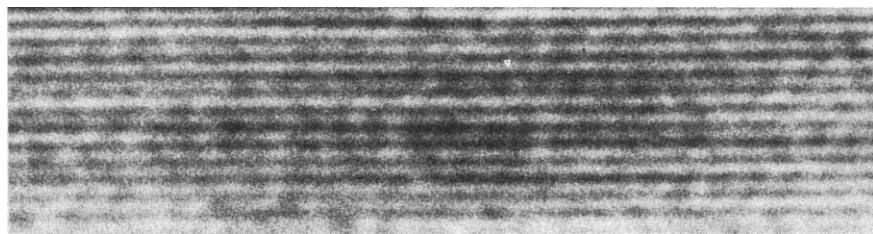
EARLY efforts to find out whether superconductivity can take place when electrons are constrained to move in only two dimensions were confined to experiments on thin films attached to a substrate by vacuum deposition. And attempts to study the effect of organic molecules on superconductivity were limited to the technique of depositing alternate layers of metal atoms and organic molecules. Both types of experiment suffered from the difficulty of properly characterizing the films.

A year or two ago, however, those interested in superconductivity began to take notice of the metal dichalcogenides—layered solids like TaS₂ that are held together by van der Waals forces. In the case of TaS₂ each layer itself comprises two layers of sulphur atoms with tantalum atoms in between.

Gamble *et al.* (*Science*, **168**, 568; 1970) were the first to try to solve

the two-dimensional superconductivity problem by separating the layers of TaS₂ with organic molecules, pyridine especially. They came to the conclusion that superconducting properties still persisted, and that the thickness of the organic layer did not have much effect on parameters such as the superconducting transition temperature.

In next week's *Nature Physical Science*, Thomas *et al.* report their elegant electron microscopic study of the expansion that follows insertion of pyridine molecules between the TaS₂ layers. The figure here shows the situation before and after insertion of a single layer of pyridine molecules. The periodicity of the layers evidently changes from 0.60 nm to 1.01 nm, a result which suggests very strongly that the pyridine molecules lie with their planes parallel to the chalcogenide layers.



× 3,900,000

strawberry latent ringspot virus. Although not causing visible symptoms of disease or distress in rhubarb, such viruses can affect the overall vigour of the infected plants and prevent them from giving their full potential yield of marketable material. In the case of rhubarb it was not possible to estimate the extent of this reduction in vigour until virus-free stocks became available in 1966-67. The results now reported are from preliminary trials started in 1967 and 1968.

Stocks of rhubarb free of viruses were obtained by meristem tip culture, a technique which has been widely used by horticultural researchers in the past few years. The tip of the meristem—the actively growing point of the plant—is cut off and cultured to produce a tiny plantlet, which grows into a larger plant. Such plants can be split up to provide clean stocks of rhubarb.

Both virus-free and infected rhubarb was grown in the two trials. When the leaves and leaf stems were harvested about two years later, yields were significantly greater from the virus-free plants. It is, of course, the fleshy leaf stems—or petioles as botanists call them—which provide the edible part of the rhubarb plant. All virus-free rhubarb planted in 1967 and harvested in 1970 gave 101 per cent greater weight of petioles per plant than did the corresponding infected plants. The difference was also easy to see, for virus-free plants were quite obviously larger and more vigorous at the end of the trial. Results were similar for rhubarb planted in 1968.

As Walkey and Cooper point out, the circumstances of their trials did not absolutely resemble commercial conditions; for one thing, they "pulled" the leaves from their plants three times in one season, which is not normal horticultural practice. But they feel that the vigour and yield of virus-free rhubarb could be considerably greater than they are at present for commercial stocks.

RNA POLYMERASE

Simple and Specific

from our Cell Biology Correspondent

IN marked contrast to the RNA polymerase of *Escherichia coli*, which is a large and complex molecule with five polypeptide chains ($\alpha_2\beta\beta'\sigma$), the RNA polymerases specified by the coliphages T3 and T7 are single polypeptide chains with molecular weights in the order of 100,000. But, as the recent experiments of Maitra and Huang (*Proc. US Nat. Acad. Sci.*, **69**, 55; 1972) emphasize, in spite of their simplicity these coliphage enzymes are at least as specific as the RNA polymerase of *E. coli*; clearly, loss of specificity has not been the inevitable

evolutionary price paid for a simple polymerase.

Maitra and Huang find that *in vitro* T3 polymerase specifically transcribes for prolonged periods only native T3 DNA; although T7 polymerase can transcribe both native T3 and T7 DNAs the T3 enzyme cannot transcribe T7 native DNA. The RNA chains made *in vitro* by the T3 enzyme have an average chain length of between 6,000 and 8,000 bases without exception they begin with a pppGp residue and as expected they compete in hybridization experiments with late T3 DNA made *in vivo*. Apart from native T3 DNA the T3 enzyme, like T7 polymerase, can also transcribe poly(dG.dC) and poly(dI.dC) to yield poly(rG), but it cannot use poly(dA-T) as a template, presumably because the latter is devoid of dC rich regions required for initiation.

When offered as template denatured T3 DNA the T3 enzyme loses its specificity; RNA chains made are much shorter, averaging 800-1,500 bases and begin either with pppGp or pppAp.

During a fourth incubation in Maitra and Huang's conditions, each molecule of polymerase transcribed from native T3 DNA about twenty-eight RNA chains. In other words the enzyme acts catalytically, recycling by repeatedly initiating, elongating, terminating and releasing transcripts. This observation must mean that as well as recognizing *in vitro* specific initiation sites the enzyme also recognizes some termination signal, stops synthesizing, releases the RNA and is itself liberated from the template. Whether the termination sites recog-

nized *in vitro* are identical to those recognized *in vivo* has yet to be established. And although addition of the *E. coli* termination factor rho has no effect on *in vitro* transcription by the T3 enzyme there remains the possibility that *in vivo* correct termination depends on the presence of a termination factor specified by the T3 genome.

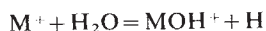
A comparison of the 3' terminal sequences of T3 RNA made *in vivo* and *in vitro* should resolve these issues, but whatever the outcome it is abundantly obvious that T3 RNA polymerase is a highly specific enzyme and one cannot avoid wondering why *E. coli* requires such a very complex polymerase when specificity can be achieved with much simpler molecules.

Phage T3 and phage T7 are very closely related and apart from specifying very similar RNA polymerases both phages inhibit the activity of *E. coli* RNA polymerase on infection. Brunovskis and Summers (*Virology*, **45**, 224; 1971) have suggested that among the early T7 proteins there is an inhibitor of host transcription, and Mahadik *et al.* (*Proc. US Nat. Acad. Sci.*, **69**, 162; 1972) seem to have partially purified from cells infected with T3 a protein which inhibits host transcription, and which may well be specified by the phage. This protein only marginally inhibits transcription by T3 polymerase, markedly inhibits transcription by *E. coli* holoenzyme ($\alpha_2\beta\beta'\sigma$) but does not inhibit transcription by *E. coli* core enzyme ($\alpha_2\beta\beta'$). This pattern of inhibition strongly suggests that the inhibitor protein acts at initiation, and in this respect resembles rifampicin, probably by antagonizing sigma factor.

Hydrogen Atom Concentrations in Flames

HYDROGEN atoms feature prominently in flame reactions so that it is always significant when a new method of determining their concentration is proposed. This is especially true because most techniques have their drawbacks and are not universally applicable. Such a new method has been investigated in the Department of Chemical Engineering at Cambridge and is reported by Hayhurst and Kittelson in next Monday's *Nature Physical Science* (February 14).

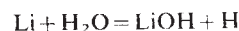
The method is based on the measurement of ion concentrations in hydrogen + oxygen + nitrogen flames, seeded with alkaline earths ($\ll 0.1$ p.p.m.), in the temperature range 1,500 to 2,500 K. The balanced equilibrium linking the concentrations of M^+ and MOH^+ is



where M can be Ca, Sr or Ba. $[M^+]/[MOH^+]$ thus gives a measure of [H]. The ions are detected mass spectrometrically and Hayhurst and Kittelson argue

that the measured ion current ratio I_{M^+}/I_{MOH^+} , though not necessarily equal to $[M^+]/[MOH^+]$ in the flame, is proportional to [H] in the flame. They have tested this hypothesis experimentally by studying the variation of the relative [H] determined by an independent optical method and of I_{M^+}/I_{MOH^+} as a function of distance along the flame axis for several different flames. The authors also show that, for many flames, [H] can be put on an absolute basis.

The new method should prove particularly useful in cases where others fail altogether. For example, the well known Li/LiOH technique involving the equilibrium



is of little use in oxygen-rich flames, or if some other substance affects the balance of the equilibrium. It is also valueless if there is interference with the emitted resonance radiation of Li atoms, as in some industrial situations.

PROTEINS

Enzymes Released

from our Molecular Biology Correspondent

A LARGE number of the enzymes of the greatest interest are confined, like a genie in a bottle, to the interior of a cell membrane, and often expire when they are liberated. An increasing number are now coming to light, however, which are active in aqueous media, or sometimes in simulated solvent conditions which approach more or less those of the membrane. The properties of some of these proteins are engrossing: a recent example is the enzyme C_{55} -isoprenoid alcohol phosphokinase (Sandermann and Strominger, *Proc. US Nat. Acad. Sci.*, **68**, 2441; 1971) which has been found to contain no less than some 60 per cent of non-polar amino-acids, and partitions out of water into wet butanol.

Another enzyme, and one that should commend itself to anybody looking for a ride on the gravy-train of socio-economic relevance, is the DDTase that occurs in resistant strains of houseflies. Because the solubility of DDT in water is of the order of 10^{-9} moles/l., it must be assumed that the DDT does its work in a lipid milieu, and that this too is where the enzyme is to be found. The location of the DDTase, or more precisely DDT-dehydrochlorinase, has not in fact been determined, but a new study by Dinamarca, Levenbook and Valdés (*Arch. Biochem.*, **147**, 374; 1971) has now established that it is a lipoprotein: a considerable quantity of phospholipid is associated with it, and it has not proved possible to extract it without denaturation.

The enzyme has a number of curious features. In the presence of a reducing agent, such as mercaptoethanol, it exists as a monomer of 30,000 molecular weight, but as prepared in the absence of thiols, it is an enzymatically active tetramer. Moreover, addition of substrate to the monomer causes it to associate into tetramers, which appear to be the only active species. At low concentrations of reducing agent the dimer and trimer can also be observed in gel electrophoresis. The protein has a high thiol content, and no disulphide bonds can be detected, even in the tetramer, which leaves the effect of the reducing agent unexplained and seemingly inexplicable. The paradox is compounded by the observation that reduced glutathione, actually a cofactor in the catalytic process, stabilizes the tetrameric form. Taking the results at face value, one can only speculate rather helplessly about thiol groups of very low redox potential.

The four subunits are probably identical, for they are homogeneous in gel electrophoresis, produce the right

number of tryptic peptides for the content of lysine and arginine calculated on a monomer basis, and have only one kind each of C-terminal and N-terminal amino-acids. DDTase is a thiol enzyme in the sense that titration with a mercurial leads to progressive inactivation. In the absence of substrate some of the thiols react quickly, others more slowly, presumably with unfolding, but when DDT is present all of them become unreactive. Dinamarca *et al.* suggest that the phospholipid associated with the enzyme has the function of dissolving the DDT, and presumably leading it to the active site. The alternative of course is that it comes from a membrane in which the enzyme resides.

A membrane protein which has attracted the attention of Kornberg's laboratory is the phospholipase A of *Escherichia coli* (Scandella and Kornberg, *Biochemistry*, **10**, 4447; 1971). This turned up in healthy cells, which were being screened as a control for phage-infected cells, on the principle that the latter might be expected to have a specific phage-induced phospholipase. Such an enzyme could open a channel in the membrane, through which the products of the phage genome, such as lysozyme, would pass. The *E. coli* enzyme is evidently embedded in the membrane, for it is released only by procedures that break down the membrane structure, and it is not susceptible *in situ* to proteolytic attack. Its specificity is unusual in that it will act on

L-phosphatidylcholine, but not on the D-enantiomer. As isolated, the phospholipase contains lipid, but this may be solvent-extracted without detriment to the enzymatic activity. The lipid-free protein, however, forms aggregates.

Among the more diverting properties of this enzyme is its ability to survive exposure to concentrated sodium dodecyl sulphate. Whether this is a matter of renaturation after removal of the detergent or of persistence of the native conformation in its presence is uncertain, however, for the alkyl sulphate is a competitive inhibitor and the assays cannot be performed in its presence. This is a finding which should persuade workers in the membrane field to moderate their faith in the infallible dissociating and denaturing capacity of the dodecyl sulphate ion.

Another type of membrane protein, evidently not an enzyme, but serving a vital function nonetheless, has been isolated from *E. coli* by Weiner and Heppel (*J. Biol. Chem.*, **246**, 6933; 1971). It binds glutamine with an equilibrium constant of 10^{-7} M $^{-1}$, and is presumed to be involved in the active transport of this amino-acid. The protein is released from the membrane into the solvent by osmotic shock. It is known that some amino-acid binding proteins are readily liberated in this way, whereas others remain associated with the membrane. The glutamine binding protein evidently retains its binding activity in aqueous solution.

The Cell Coat and Transformation

ANY parameter of the cell surface which can be measured or assayed has attracted great attention recently, for it is clear that the transformation of non-malignant cells to malignancy, either by tumour viruses or by chemical carcinogens, is accompanied by profound but subtle changes in the chemistry and architecture of the cell surface. One of the few parameters of the cell surface that can be measured by physical methods is the thickness of the cell coat. But as the ellipsometric measurements, reported by Mallucci *et al.* in *Nature New Biology* next Wednesday (February 16), indicate, any hope that a change in the thickness of the cell coat might prove to be a consistent feature of transformed cells must be forgotten.

As Mallucci *et al.* have found, mouse cells isolated from a tumour induced by polyoma virus have a coat at least twice as thick as that of untransformed mouse embryo fibroblasts but the coat of mouse cells isolated from tumours induced by methylcholanthrene is not significantly thicker than that of untransformed cells. Moreover, the rate at which virus transformed and chemically transformed cells resynthesize

their coat after exposure to trypsin differs markedly, although in both cases it is faster than the rate in untransformed cells.

This resynthesis of coat glycoprotein is blocked to an equal extent in untransformed and both sorts of transformed cells by cycloheximide but the response of virus transformed and chemically transformed cells to actinomycin D is very different. Although actinomycin D rapidly inhibits RNA synthesis and coat synthesis in untransformed and chemically transformed cells, the cells transformed by polyoma virus continue to make coat material for 100–220 minutes after exposure to the drug and after RNA synthesis has been more or less totally inhibited. It seems therefore that in the virus transformed cells, but not in the chemically transformed cells, the messenger RNAs with specific coat materials may have enhanced stability. These findings together indicate that as far as the thickness and synthesis of the cell coat are concerned, virus transformed and chemically transformed cells differ from each other at least as much as they differ from untransformed cells.

LASERS

Heating Deuterium

ONE attractive proposal for a thermonuclear reactor that has emerged during the past few years has involved the heating of a solid pellet of deuterium, or deuterium and tritium, with a laser beam, so that a thermonuclear reaction occurs during the very rapid expansion phase. Apart from the intriguing problem of how to operate a trapdoor arrangement at such a speed that the laser is not demolished by the thermonuclear shock-wave, one serious drawback of the idea is that the pellet might not absorb energy and that available lasers might not be sufficiently energetic (see J. L. Tuck, *Nature*, **233**, 593; 1971).

One of the stumbling blocks has been that a deuterium-tritium pellet at 4 K, for example, has a density of 6×10^{22} atoms cm^{-3} , which suggests that the plasma frequency would be so high that no laser radiation could be absorbed. But in a recent issue of *Physical Review Letters* (**28**, 85; 1972), R. P. Godwin of the Los Alamos Scientific Laboratory presents a model to explain recent experimental indications that total reflexion is not always the inevitable outcome of shining a laser beam onto a deuterium pellet.

The stimulus for Godwin's work came from a "workshop" on laser interactions which was held last September at the Rensselaer Polytechnic Institute (the proceedings of which are to be published). At that gathering, F. Flux reported that he had observed energy absorption of 64 per cent when a 44 J Nd-glass laser pulse was fired at a deuterium pellet; this absorption was accompanied by the production of neutrons, X-rays and energetic ions. Other work reported at the conference last September also suggested that it was necessary to administer a small amount of energy to the laser target just before the laser pulse proper (in the form of a prepulse or of a shaped leading edge for the main pulse).

Godwin has suggested that the absorption can be understood in terms of a model in which the plasma sample has on its surface a small amount of plasma with a low density and a plasma frequency equal to the laser frequency; the remainder of the sample, he postulates, has the high density and high plasma frequency expected for a deuterium pellet. Godwin first performs some calculations on a system beloved of the teachers of undergraduate courses in electromagnetic theory—a thin film with polarized radiation impinging upon it at an angle. The assumptions made in this case are that the thin film is a plasma, the density of which is such that the frequency of the electromagnetic radiation is equal to the plasma frequency, and that the

complex dielectric constant is that of a slightly damped Drude free electron gas. It turns out that the maximum absorption under these circumstances is 50 per cent and occurs when the thickness of the film is half of the wavelength of the electromagnetic (laser) radiation.

If such a film is now imagined to be backed by plasma of higher density, which acts like an almost perfect conductor and therefore like a mirror, the electrostatic method of images leads one to the conclusion that maximum absorption (100 per cent this time) should occur when the film is a quarter of a wavelength thick. (For unpolarized laser light, of course, the maximum absorption is only 50 per cent.)

Godwin presents this "hot spot" model as the basis for an understanding of how Flux has managed to achieve absorption of laser radiation in a solid deuterium sample. Plainly, the use of a precursor pulse is the crux of the matter, for it provides the energy both to ionize atoms on and near the surface and to increase their average separations so that the electron density is $\sim 10^{21}$ cm^{-3} , the critical value for which the plasma frequency is equal to the frequency of the light from an Nd-glass laser.

As far as the maintenance of a thermonuclear reaction is concerned, of course, it is the energy available to the deuterium (or deuterium and tritium) ions that is important. Under ideal conditions and with a laser pulse energy of 10 J, Godwin calculates that the energy available for each particle is about 10 MeV. But lest anybody should think that controlled thermonuclear

fusion on a practical scale is just around the corner, he hurriedly qualifies this by saying that when energy loss mechanisms such as thermal conduction are taken into account the particle energies will certainly not be in the MeV region. He does not, however, make an estimate of what the particle energies will be. In his article in *Nature*, Tuck mentioned threshold temperatures of about 10 keV for deuterium-tritium thermonuclear reactions and about 50 keV for those between deuterium and deuterium.

PESTICIDES

Mosquito Control

from a Correspondent

NEARLY all methods of mosquito control in operation at present are based on the use of insecticides. In spite of the growing problem of resistance to chlorinated hydrocarbons, organophosphates and to carbamates the campaigns against mosquito-borne diseases still depend on the use of residual insecticides generally and DDT in particular. Sanitary measures of reduction of breeding sites are not economically feasible in rural areas of developing countries and biological control offers some promise but little immediate help. Thus any withdrawal of insecticides from public health use would jeopardize nearly all public health programmes aiming at the eradication or control of malaria, yellow fever, dengue, virus encephalitis and filariasis—all mosquito-borne diseases. These were the principal points of a symposium on problems of mosquito control convened in London on January

Two-headed Enzyme from Horse Muscle

AS part of a long-term programme of structure determination of all the enzymes in a metabolic pathway, Blake, Evans and Scopes have studied the glycolytic enzyme, phosphoglycerate kinase, from horse muscle, and its structural features at 6 Å resolution are reported in next Wednesday's *Nature New Biology* (February 16). The protein is a single polypeptide chain with the rather unusually high molecular weight of 48,000. The electron density maps show that it is in effect made up of two globular subunits, joined together by a narrow neck. The globular portions correspond in size to a molecular weight of some 20,000 each, one being elongated, the other nearly spherical. This leaves 8,000 for the neck, which is about 20 Å thick, and in which helix-like structure can be made out.

In an attempt to locate the active centre, magnesium and ATP, one of the substrates, were allowed to diffuse

into the crystals. This, however, proved unsuccessful, for the crystal cracked, indicating that a conformational change in the enzyme had occurred. With magnesium and ADP, on the other hand, isomorphous patterns were obtained, and the electron density difference map showed only a single area of positive intensity, corresponding to the magnesium-ADP.

The profile has two lobes, which are surmised to represent the nucleoside and the magnesium-diphosphate parts of the magnesium-ADP complex. One of the lobes dips into a shallow depression in the protein, and the other into the solvent. The binding site is in the more elongated of the two globules and so far no function for the other subunit has been found. The speculation is that it has either a modifying function on the activity, or that it is implicated in an interaction with some other enzyme in the pathway.

17 by the Pesticides Group of the Society of Chemical Industry.

Professor J. D. Gillett (Brunel University), having discussed the patterns of behaviour of vectors in relation to the transmission of human disease, pointed out that isolated populations or laboratory colonies of mosquitoes are cut off from the total gene-pool of the species which has a plasticity and resilience that assure its survival in many adverse conditions. Professor J. R. Busvine (London School of Hygiene and Tropical Medicine) analysed the chief trends in the appearance and spread of resistance in anopheline and culicine mosquitoes and indicated the reasons for different rates of increase of resistance in the two.

Dr A. B. Hadaway (Chemical Research Establishment, Porton Down) described the World Health Organization's programme for testing and evaluation of new insecticides which, having screened some 1,500 candidate compounds, selected five for final field trials. Mr F. Barlow (Chemical Research Establishment, Porton Down) outlined the ways in which physical and chemical properties of insecticides influence their performance in the field. Dr W. W. Macdonald (Liverpool School of Tropical Medicine) discussed the evaluation of control methods in the field and pointed out their dependence on the species of mosquito and on environmental conditions.

Dr J. M. Barnes (MRC Toxicology Unit, Carshalton) considered the toxicity of public health insecticides for spraymen and for the general population. He stressed that in spite of heavy exposure to DDT for a number of years there is no evidence of short or long term adverse effects on either of the two groups. Most of the new, rapidly biodegradable insecticides have higher initial toxicity than DDT and require more cautious operational safety measures.

Dr G. Davidson (London School of Hygiene and Tropical Medicine) considered the alternative measures for mosquito control and stressed the potential value of methods involving genetical manipulations, namely the release of male mosquitoes deliberately sterilized by irradiation or chemosterilants, sterile hybrid mosquitoes produced by crossing closely-related species, the introduction of cytoplasmically incompatible individuals or of individuals possessing chromosomal translocations.

Mr J. W. Wright's (WHO) contribution, read in his absence, outlined the world wide importance of mosquitoes as carriers of malaria, yellow fever, dengue, filariasis or viral encephalitides and the situation that might arise if the public health use of insecticides is seriously affected.

MEDICAL RESEARCH

Tumour Progression

from a Correspondent

STUDENTS of the growth of tumours have for many years recognized that the rate or quality of the growth seems to change with time. Some of the observed changes have been described as indicative of tumour progression, defined by Foulds as "Progression is . . . a change in a portion of an old lesion . . . establishing a new tumour having properties not formerly manifest". On January 24 in the British Museum (Natural History) the third of the meetings organized by the Institute for Cancer Research on the principles of clinical and experimental oncology considered this topic.

The chairman, Dr F. J. C. Roe (Tobacco Research Council), said that progression should be recognized as a phenomenon especially by those people engaged in short-term experiments on "bits of tumours or bits of animals", otherwise variation in their results might assume undue significance. Dr Roe went on to stress that neither growth *per se*

nor alteration of the host, affecting tumour growth, should be thought of as progression. In the experimental induction of tumours it is also quite likely that changes could occur in two cells or cell populations giving rise to tumours with different periods of latency. The emergence of a second, qualitatively different cancer could then easily be confused with progression. Dr Roe cited as an example of progression the development of mammary tumours in mice during which hyperplastic nodules with variable timing give way to hormone dependent tumours and eventually hormone independent tumours could arise. It was stressed that, particularly in relation to lung tumours, the definition of progression is exceedingly difficult to apply on strict histopathological criteria.

Dr A. Glucksmann (ARC Institute of Animal Physiology, Babraham) described various changes which could take place in experimentally induced tumours, stressing that a variety of both exogenous and endogenous factors could influence growths found by the histopathologist. He felt that invasiveness *per se* should not be

Immunoglobulin Secretion and Cytochalasin B

IF cells are incubated with an inhibitor of known activity and they continue to carry out some function, it is possible that the function is insensitive to the inhibitor or alternatively that the wrong concentration of inhibitor has been used or even that the inhibitor only works in certain environmental conditions which have not been met in the experiment. Parkhouse and Allison, in next Wednesday's *Nature New Biology* (February 16), publish an interesting account of the failure to inhibit secretion of immunoglobulins from plasmacytoma cells by cytochalasin B, an inhibitor of the microfilament-related contractile systems in many cells.

Parkhouse and Allison first review the release of granular packages of secretions from cells; cells from the medulla of the adrenal release catecholamines on stimulation by acetylcholine, insulin containing granules emerge from pancreatic β -cells exposed to high concentrations of glucose, and mast cells release granules containing histamine, serotonin and slow-reacting substance when brought in contact with cell-bound antigen-antibody complexes. Plasma cells, however, are different in that they seem to release their secretory products more or less continuously without further external stimulus. Nevertheless it has been suggested that immunoglobulin is packaged in small vesicles which discharge their contents by reverse pinocytosis (J. W. Uhr, *Cell Immunol.*, 1, 228; 1970).

Parkhouse and Allison therefore elected to attempt to suppress the release of immunoglobulins from cells of a plasmacytoma by cytochalasin B, which it has been shown can suppress granule release from mast cells (T. S. C. Orr, A. C. Allison and D. E. Hall, *Nature*, in the press). Their method involved incubation of tumour cells with radioactive leucine. Radioactive immunoglobulins were recovered from the supernatant after precipitation with appropriate heterologous anti-immunoglobulin antisera. Cytochalasin B had little effect on the amount of recovered immunoglobulin. Neither did colchicine which is said to disperse labile cytoplasmic microtubules.

If these results can be substantiated using normal immunoglobulin secreting cells, such as in a Jerne plaque method, and with a wider range of concentrations of inhibitor, it will seem that there are at least two methods of secretion by cells, the first typified by mast cell degranulation, the second by plasma cells. The property that some lymphocytes, and perhaps all cells, have to remove materials from their surfaces by cap formation (R. B. Taylor *et al.*, *Nature New Biology*, 233, 225; 1971) was not drastically affected by cytochalasin B, though no experiments were made to discover whether the re-establishment of receptors on the cell surface was affected. If immunoglobulin secretion is involved it now seems likely that cytochalasin B will not have an effect.

thought of as a reliable characteristic of malignancy but that the added attribute of xenoplasia (ability to grow in a foreign environment) should be taken into consideration. He illustrated this concept by showing several instances in which tumour growth had resulted in the rupture of basement membranes in host tissue but that cells broken off from the tumour mass appeared unable to survive in the newly invaded environment.

Professor J. R. Hobbs (Westminster Hospital, London) in a lucid and well illustrated talk discussed observations that have been made on paraprotein levels in mice and men with immunocytomas. Professor Hobbs said that the term immunocytoma had been adopted to cover abnormal growth of cells which produced immunoglobulins, irrespective of their morphology. He adduced the evidence that paraproteins are produced from clones of cells all secreting recognizably the same product. He failed seriously to bear in mind that informational transfer between cells could give rise to cells which all secrete the same immunoglobulin. At present, however, such notions are suspect and Hobbs's opinion about the monoclonal nature of paraproteins is widely shared. Professor Hobbs established that in mice and men the amount of paraprotein detectable in the serum could be used as an index of tumour growth. He then went on to examine the concept of progression in relation to immunocytomas.

Three kinds of untreated condition are broadly distinguishable. Those in which the paraprotein level though abnormally high was stable, those in which an exponential increase with time was inexorable and those very rare cases in which a previously stable situation progressed to an increasingly abnormal one. Professor Hobbs, however, went on to show that in 45 per cent of all treated cases the condition before treatment and that afterwards could be qualitatively different in a variety of ways. The change, he felt, might well be attributable to the mutagenic effects of the treatment, particularly in those instances in which the new paraprotein showed only a single amino-acid change from the previous one.

ANIMAL COLORATION

Jumping for Life

from a Correspondent

THE phenomena of "warning" (aposematic) and "mimetic" (pseudaposematic) colouring in animals have been dealt with in the classic work of H. B. Cott (*Adaptive Colouration in Animals*, Methuen, 1940) and more recently by E. Wickler (*Mimicry in Plants and Animals*, Weidenfeld and Nicolson, 1968). Animals which are poisonous,

distasteful or otherwise inedible are apt to have conspicuous and characteristic colour patterns, usually in black and yellow or black and red, which may be mimicked by other, more "edible" species.

In a recent article (*Entomol. Scand.*, 2, 41; 1971), C. H. Lindroth suggests that patterns of the "warning" type may be borne by species whose only defence is the power of jumping in response to a visual or tactile stimulus. Birds, he suggests, may learn to recognize and to avoid species which habitually frustrate them by jumping just before they can be seized. The examples he considers are drawn from a well known group of jumping insects, the "flea-beetles" (Chrysomelidae-Alticinae). Some species of this group, for example, the South African *Diamphidia* and *Polyclada* species used for making arrow poisons, are definitely poisonous and have typical "warning" colours; these species hardly jump at all. In other genera, for example, the American *Disonycha*, species showing similar types of colouring are strong and ready jumpers and Lindroth quotes evidence that they are not distasteful or poisonous to birds.

Lindroth suggests that these *Disonycha* species have served as models for mimics; the ones he cites are carabid beetles of the genus *Lebia*, whose larvae are known or believed to

develop parasitically on the pupae of the very same Alticinae which the adults mimic. The adult *Lebia* are apt to occur on the foodplants with their *Disonycha* hosts, in relatively small proportions—so the prime requirement for successful Batesian mimicry, that the mimics should be less common than their models, is likely to be fulfilled.

If Lindroth is right about the significance of the colouring of these flea-beetles, it is likely that similar phenomena will be displayed in other jumping animals. Among vertebrates, the most likely group to show them is Anura. The agile African tree-frogs of the genus *Hyperolius* are diurnally active and liable to show aposematic-type patterns (for example, *H. marmoratus* figured by Cott). Among British insects, Alticinae are probably rather too small to make effective use of warning patterns (there are few cases of animals less than about 4 mm long with colouring which is definitely aposematic), but at least one species of the homopteran bugs, the cercopid *Triecphora vulnerata*, is of comparable size with the *Disonycha* species, a strong jumper, and is strikingly marked in bright red and greenish black. It would be interesting to make a particular study of this species with Lindroth's theory in mind.

Latent Herpes Simplex Infection

ONE of the most fascinating aspects of the biology of the herpes viruses, and of herpes simplex virus in particular, is their capacity to persist in their natural hosts in the face of circulating antibodies and periodically to erupt. Many people infected with herpes simplex, for example, periodically and often predictably suffer from recurrent herpetic infections in response to physical or emotional stimuli and the resulting lesions always appear in the same part of the body. Clearly, this virus must persist in infected individuals, but which cells harbour the virus between eruptions? Much indirect evidence has been accumulated which incriminates the sensory ganglia and cells associated with nerve trunks as the reservoirs of the virus; the experiments with rabbits reported in next Wednesday's *Nature New Biology* (February 16) by Stevens, Nesburn and Cook are more than consonant with that idea.

Stevens and his colleagues find that trigeminal ganglia taken from rabbits a few days after the animals have been infected by dropping suspensions of herpes simplex virus onto the cornea of the eye contain from 70 to 7,200 plaque forming units of the virus per gram of tissue. Clearly, after infecting the eye the virus somehow reaches and infects

the trigeminal ganglia. Stevens *et al.* therefore maintained rabbits with recurrent ocular herpetic infection for 4 to 8 months, daily assaying the tear film for herpes simplex virus. As expected the virus intermittently appeared and disappeared from the tear film; virus could not, however, be detected in, or recovered from, trigeminal ganglia taken from such rabbits. But when portions of trigeminal ganglia were put into organ culture herpes simplex virus could eventually be recovered from at least some of the cultures.

It seems therefore that the virus can latently infect the trigeminal ganglia and can be induced to replicate to yield detectable amounts of virus when the latently infected cells are cultured *in vitro*. Precisely how the viral genome persists in the latently infected cells remains to be discovered; it may replicate to yield very small amounts of virus which are undetectable with current assay procedures or the viral genome may persist in a repressed state. But, that apart, if herpes simplex virus can latently infect sensory ganglia of rabbits the same may very well be true of man; in other words, sensory ganglia are probably the site of the reservoir of herpes simplex virus in persistent infections of man.

Why do they Leave?

SUSAN LIPSHITZ

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This article considers some aspects of dropping out and wastage of students from craft courses.

LARGE numbers of students "drop out" of courses each year in higher and further education, and are seen as "wastage" of energy and resources that the individual and society have invested in training and education¹. As the engineering industry is the largest employer in Britain, influencing much training directly and indirectly, the data refer only to engineering courses. This article deals only with wastage from first year craft level courses in the further education system which contributes part of the national loss from technology courses.

In Britain, engineering is usually seen as a typically masculine occupation and few girls enter the field, so my remarks about the wastage process in this context apply chiefly to boys. Until now wastage rates for one particular college could not be put into general perspective because there seemed to be no figures collected nationally on the same basis, and in a limited way this article is an attempt to remedy that lack.

Generally the rate of loss of entrants to education courses before they qualify has been shown to vary between institutions, though remaining fairly constant for one institution². Evening and part time courses lose more students than full time day courses³. Although some reasons for dropping out, like tensions at examinations, may be common to all types of training and education, others may be specific; for example, students in full time residential institutions⁴ will have some problems that differ from those of craft course students who start their course younger, are work based and usually live at home. Drop-outs at different stages in a course may also leave for different reasons, and so it is important to make clear the distinction between the terms drop-out and wastage.

For some drop-outs from college, contact with the institution may have been beneficial if it helped them to discover interests they would rather pursue; it may even cause them to "drop in" somewhere else. Wastage, on the other hand, comprises those drop-outs for whom leaving a course in further education represents their final contact with the entire education system. As they often simultaneously leave their jobs, many also abandon the opportunity of being trained in an industrial skill. These distinctions are not clear until students are followed up, as was done in a small depth pilot study which I recently completed. A group of motor vehicle craft apprentices who had left their college course by the end of their first two terms were compared with boys still on the course. About two-thirds of the drop-outs were also wastage a year after leaving—that is, they had unskilled jobs—although the rest had jobs with appropriate training as apprentices or trainees. Some were still in engineering and some were in other work. Interviews showed that fewer drop-outs (including wastage) had expected to go to college as part of their training. They also regarded the college atmosphere as very similar to that of their schools, where they had experienced academic failure more often than boys who stayed on the course. It seemed that these influences were important in making the first year, even

Table 1 Geographical Distribution of the National Sample of Colleges providing Craft Level Engineering Courses

Area	No. of colleges
North	1
East and West Midlands (combined)	1
Yorkshire and Humberside	3
East Anglia	0
Greater London	2
Other South East	5
South West	3
North West	1
Wales	1
Scotland	3
Northern Ireland	0
Total	20

before any question of examinations arose, a period of high risk for students dropping out. Some boys leaving school and settling down to work will inevitably become dissatisfied with some aspects of their situation and will leave it. To the extent that it is impossible to eliminate unsuitable choices of work by the school leaver, or changes occurring in his interests, or redundancy, it is also impossible to eliminate dropping out at this and later stages. Later on in a course, however, students are more likely to leave for other reasons, for example they need more money than they get as apprentices or trainees, in order to get married. Although they may not have gained their academic qualifications, they may have already achieved skilled status at work and so might be able to get a job above the unskilled level. Thus they might be drop-outs but not wastage in terms of their personal and industrial situation, although they are wastage from the point of view of the education system. Obviously industrial factors are very important here, as employers often instigate a boy's attendance at college, and their training policy and interest will largely determine a boy's success and whether he leaves the course or not. To the extent that the present system of training and education is inflexible and unwilling or unable to make apprenticeships transferable, or the accumulation of course credits over a long period of time possible, some dropping out is inevitable. These are some of the influences likely to underlie the wastage process in the context described in the figures presented here.

The data were provided by a national sample of twenty colleges comprising a 10 per cent random sample of all colleges in the United Kingdom who entered students for examinations set during a mechanical engineering craft course (193) run by the City and Guilds in 1970. Three types of courses were covered—mechanical craft, motor vehicle and auto craft, and "all other engineering" craft, which includes shipbuilding, fabrication and welding. Data are available for two course years which may indicate fluctuations in the entry and wastage rates for each college; although figures for only two years cannot be very reliable.

Table 2 Contributions of the Different Courses to the National Craft Course Entry

Course	1968/69 (%)	1969/70 (%)
Motor vehicle	31	32
Mechanical craft	45	47
All other engineering craft	24	21

Table 3 Entry and Wastage for Craft Courses

Year and course	Entry numbers	Number of students remaining at end of first term	Number of students remaining at end of first year (before exams)	% loss from course by end of first term	% loss from course by end of first year
1968/69					
Motor vehicle	973	852	805	-12	-17.25
Mechanical craft	1,420	1,232	1,247	-13 *	-12
All other engineering craft	752	598	627	-20 *	-16
Total	3,145	2,682	2,679		
1969/70					
Motor vehicle	1,006	918	845	-8.7	-16
Mechanical craft	1,501	1,262	1,319	-15.9 *	-12
All other engineering craft	668	599	560	-10.3	-16
Total	3,175	2,779	2,724		

* Fluctuations because of late additions to the courses.

Table 1 describes the sample in terms of the geographical areas covered and the number of colleges in each. Northern Ireland and East Anglia are the only areas not represented, but as they are not highly industrialized, this should not greatly bias the sample.

The size of the engineering departments in the colleges ranges from less than 500 to more than 1,500 students, and the proportion of craft students in each department varied from less than 35 per cent to more than 70 per cent of all the engineers. Only three colleges had more than 70 per cent craft students, as most commonly there were between 35 and 70 per cent in departments of 500 to 1,000 students covering seven different areas. So the provision of craft courses seems to be linked with a fairly large department, of which a large proportion are craft students (Fig. 1).

The number of students who dropped out during the year ranged widely between the colleges, from 0 to 50 per cent of the intake for all three kinds of course, although the rate of loss for the mechanical craft courses was lowest during the year. Mechanical craft courses were run by all twenty colleges; they contributed the largest number of students nationally (Table 2), and also accounted for 40 per cent of the total national losses in both years.

Motor vehicle courses contribute fewer students to the total entry, partly because only fourteen colleges in the sample ran these courses, but they nevertheless still account for 36 per cent of the national losses during the year. This is almost as much as the mechanical craft courses lose because the motor vehicle courses lose proportionately more students (see extreme right hand column in Table 3). Fifteen colleges ran courses in the "other engineering craft" category that together accounted for 25 per cent of the total national losses up to the first year examinations.

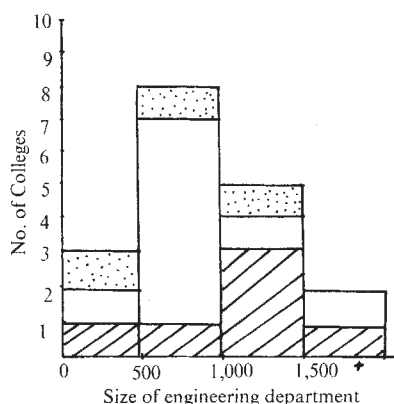


Fig. 1 Proportions of craft students by size of department. Stippled areas, 70%+; white areas, 35-70%; hatched areas, up to 35%.

A differentiation was made between the numbers of drop-outs by the end of the first term and by the end of the year because, if first year dropping out and wastage are related more to the situation experienced before college entry (for example, the firm or works suitability), then the chief losses might be expected to occur early in the year. Table 4 tends to bear this out.

Table 4 Loss in the First Term in Relation to the Total Loss

Year of course	Numerical loss in the first term	Numerical loss in the first year	Loss in the first year as % of entry	Loss in the first term as % of total loss
1968/69	463	466	11	99.9
1969/70	396	451	14	87.7

The drop-out rate by course as a percentage of the entry to that course is shown in Table 3; there is an unexpected trend as the rate for the whole year is lower than that for the first term in some cases (marked with an asterisk). This is due to fluctuating numbers on the courses because of late additions to the classes and raises the important question of whether these and other figures are adequately defined. For example, it is not known how many of the same individuals were in both the entry and the expanded class. The movements of individuals between classes as well as from one college to another, or out of the further education system, have to be traced if the distinction between dropping out and wastage is to be clear. This still needs to be undertaken on a national basis and is something for which most college staff do not have time.

If one can extrapolate from these data, it does seem that most dropping out occurs in the first term as expected, and that it represents movements of about 10 to 15 per cent of the craft entry. It may occur for the reasons associated particularly with a period of change for the students, as suggested at the beginning of this article, and would reinforce the arguments for more guidance facilities in schools and colleges. Greater flexibility of apprenticeships or other training contracts could guard against unsatisfactory choices being irrevocable. As industry is unlikely to see the ensuring of an individual's satisfaction as a principal part of its function, this evidence would seem to increase support for Crowther's proposal⁶ that 15 to 18 year olds should be college based and not left to industry to dictate.

¹ Vaizey, J., *Univ. Quarterly* (spring, 1971).

² Malleson, N., *New Society* (May 2, 1963).

³ Cantor, L., and Roberts, I. F., *Further Education in England and Wales* (Routledge and Kegan Paul, 1969).

⁴ Ryle, A., *Student Casualties* (Lane, 1969).

⁵ N.Y.E.C. Report "At Odds" (October 1970).

⁶ *15 to 18* (Crowther Report, HMSO, 1960).

Solution-transfer, an Important Geological Deformation Mechanism

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Several features common in sedimentary rocks which have been subjected to gravitational compaction or mountain-building movements can be explained in terms of "solution-transfer", that is, redistribution of minerals by "pressure-solution" and "crystallization" (sometimes known as "Riecke's principle"). A physical explanation of the process based on non-hydrostatic thermodynamic theory is presented here, drawing attention to examples in tectonically deformed rocks.

"PRESSURE-SOLUTION" is the phenomenon of dissolution of minerals under non-hydrostatic stress. It has been shown to be responsible for stylolite formation, and compaction, indentation and "welding" of granular material particularly in limestones, dolomites and sandstones¹⁻³. This pressure-solution occurs on surfaces statistically perpendicular either to an axis (σ_1) of gravitational load (diagenetic pressure-solution) or to an axis (σ_1) of weak tectonic compression (tectonic pressure-solution). I define σ as the normal stress and σ_1 , σ_2 and σ_3 as maximum, intermediate and minimum principal compressive normal stresses, respectively.

"Crystallization" may take place in pore spaces or in fissure cavities causing cementation of porous rock⁴ or the appearance of veins with semi-euhedral crystals, respectively. In these cases $\sigma = p$ at the crystallizing surface (where p = fluid pressure) and ordinary hydrostatic theory applies. Here, attention will be restricted to the problem of crystallization in essentially non-porous rock, which like the case of pressure-solution involves non-hydrostatic theory and requires an explanation of how phase changes and material migration can occur in solid rock.

Physical Conditions

I assume that in non-porous rock "transfer" of material from the site of pressure-solution to the site of crystallization is effected chiefly by diffusion (not bulk flow) of solute ions in an aqueous intergranular "dispersed phase" (ref. 5) or "solution film" (ref. 6), that is, along grain boundaries. This aqueous medium is certainly not a crystalline phase; hence it will be called a fluid, though it may not have all the properties of a true fluid. Its existence has long been postulated to account for the feasibility of certain metamorphic mineral reactions, particularly hydration and dehydration⁷. Indeed, relicts of ancient saline solutions are frequently preserved in microscopic fluid inclusions⁸. Also, by analogy with metals⁹ it may be inferred that grain boundary diffusion greatly predominates over intracrystalline diffusion in rocks well below their melting point, that is, in diagenetic or low grade metamorphic conditions.

Over a small area of grain boundary the normal stress is σ . p is almost certainly an independent variable, so that in general $p \neq \sigma$, and σ is equivalent to p plus a direct intercrystalline stress ($\sigma - p$). Previous non-hydrostatic theories¹⁰⁻¹³ are

restricted by the assumption that either $p=0$ or p = a constant or $p=\sigma$, and usually not much consideration has been given to the problem of solution-transfer.

Reversible Equilibrium

The following theory specifically covers the case of reversible equilibrium (equilibrium of Gibbs's free energy) between crystal and solution, on the lines indicated above, at any particular part of a grain boundary. Simplifying assumptions are that $dT=0$, no chemical reactions occur, the crystal is not a solid solution and is approximately incompressible. The crystal and its solute are considered as one component and the solvent (with or without impurities) as a second component.

Accordingly¹⁴

$$\left(\frac{\partial \mu}{\partial x}\right)_{T,p} dx = v_1 d\sigma - v_2 dp \quad (1)$$

where T = absolute temperature; v_1 = molar volume of crystal phase; v_2 = partial molar volume of solute in solution (assuming solvent partial molar volume constant); x = mole fraction of solute in solution; and μ = partial molar chemical potential of solute in solution.

The equilibrium mole fraction of solute, x (a function of concentration or solubility), increases with normal stress on the crystal and decreases with fluid pressure. Elastic changes in v_1 and v_2 will also affect x , but this effect is negligible compared with the right hand side terms of (1). Thus in an aggregate of stressed crystals x will be non-uniform because of variations in σ and/or p , so the system as a whole will be in a state of potential disequilibrium. On re-writing (1) in the form

$$\left(\frac{\partial \mu}{\partial x}\right)_{T,p} dx = v_1 d\sigma_A + v_1 d\bar{\sigma}_A - v_2 dp \quad (2)$$

with $\bar{\sigma}_A$ = average mean stress $([\sigma_1 + \sigma_2 + \sigma_3]/3)$ over one crystal and σ_A = deviatoric stress $(\sigma - \bar{\sigma}_A)$, it is seen that stability differences will arise between different faces of a single crystal, because of deviatoric stress differences, and between different crystals under different mean stress. Fluid pressure gradients could modify either situation, and uniform changes in p would affect the magnitude of any pre-existing stability difference.

The condition for crystal growth is that $x > x_{\text{equilib}}$, and for dissolution that $x < x_{\text{equilib}}$. Diffusion will cause solute to leave regions of high x (σ_1 faces and crystals under high $\bar{\sigma}_A$ or low p), thus lowering x and causing dissolution there, and migrate towards regions of low x (σ_3 faces and crystals under low $\bar{\sigma}_A$ or high p), thus raising x and causing crystal growth there. The result is a continuous process of dissolution, migration and growth, that is, solution-transfer, maintained by and tending to remove heterogeneities and/or anisotropies of stress. The strains which necessarily result, differential strain or external shape change of crystals caused by the differential stress, and volume change caused by heterogeneous $\bar{\sigma}_A$ or p , are permanent because diffusion is a spontaneous irreversible reaction.

Because diagenetic pressure-solution can occur under differential stresses lower than those required for most other rock deformation mechanisms (for example, lower than the calcite twinning yield stress and quartz brittle or plastic strength), solution-transfer should be prevalent also in tectonic conditions. The same conclusion is reached when cleavage, syn-

tectonic crystal growth and mineral differentiation are examined in the light of the foregoing theory.

Cleavage

It is well known^{15,16} that fossils and oolites in deformed rocks are often dissolved in the direction of compression and have syntaxial overgrowths (pressure-shadows) in the direction of extension (as in Fig. 1). It is not so well known that pressure-solution, on soluble minerals such as calcite, dolomite or quartz, frequently occurs on discrete undulating surfaces. As with diagenetic stylolite surfaces, dark insoluble residue consisting of clay, micas, carbonaceous matter or heavy minerals accumulates on these surfaces.

On losing the mechanical support hitherto afforded by soluble minerals, the platy residual minerals such as clay and mica take on a strong alignment or preferred orientation parallel to the pressure-solution surface. Thus pressure-solution can produce the dark seams (a conclusion also reached by Plessmann¹⁷) and the platy mineral alignment responsible for very common planes of weakness known as "fracture" cleavage, crenulation cleavage and slaty cleavage. Pressure-solution in slates, although not always as visible as in Fig. 1, must be more pronounced than in coarse-grained rocks because of shorter diffusion paths and consequent higher rates of solution-transfer.

Veins and Pressure-shadows

The corollary of pressure-solution is redeposition or crystallization (of calcite, dolomite, quartz and occasionally chlorite). In orogenic conditions this material usually crystallizes in veins and pressure-shadows.

According to the above equations, solute will diffuse towards σ_3 faces where x is least. The tendency towards equilibrium will cause the excess solute brought in to be precipitated on the σ_3 faces, and theoretically this may occur independently of the value ($\sigma_3 - p$). Redeposition does not therefore necessarily require the presence of an open cavity; it can make room for itself in solid rock; veins and pressure-shadows may expand against σ_3 because of the supersaturation induced by pressure-solution in adjacent areas.

Redeposition need not be confined to σ_3 faces provided the solution is supersaturated. But the direction of growth will generally be parallel to the σ_3 axis because least work is done against the external surroundings when expansion occurs in this direction. This explains why crystals of syntectonic veins or pressure-shadows are often fibrous and elongated parallel to the extension axis. If the stress orientation changes during growth, the growth direction will also change and



Fig. 1 External shape change of crinoid ossicles (single calcite crystals) by pressure-solution truncation at cleavage surfaces (dark wavy lines), and calcite pressure-shadow overgrowths (white). Same process on smaller scale in rock matrix. This cleavage attributed to pressure-solution. Liassic calcareous slate from Leytron, Valais, Switzerland.

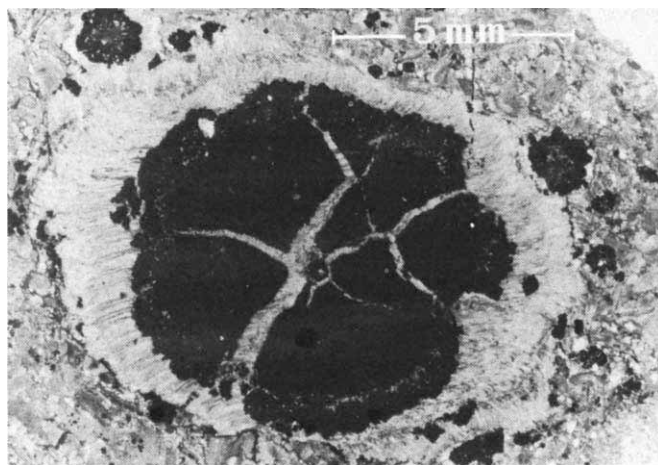


Fig. 2 Pressure-shadows and veinlets (light) with symmetrically curved fibrous calcite growths against pyrite concretion (black). L. Cretaceous biopelsparite limestone from Pont de Nant, Vaud, Switzerland.

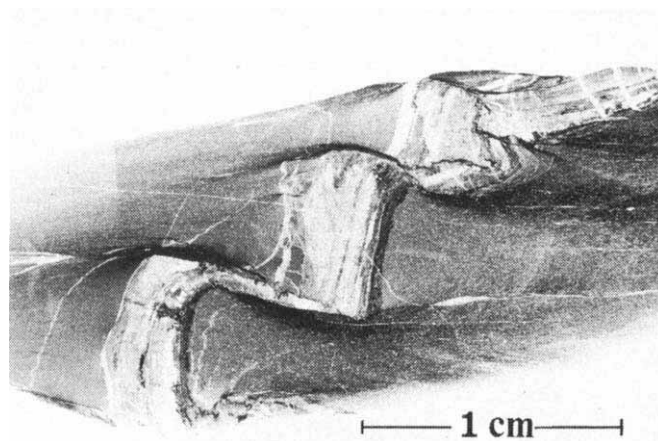


Fig. 3 Mineral differentiation adjacent to buckled calcite layer. Strong development of innumerable micro pressure-solution surfaces (cleavage surfaces) in dark regions, predominant crystallization of calcite and quartz in light regions. Calcite layer also partially dissolved next to dark regions. Flysch slate from Derborence, Valais, Switzerland.

result in sigmoidally curved fibres such as those in Fig. 2.

It seems probable that numerous tiny inconspicuous syntaxial pressure-shadows would account for more redeposition than veins or conspicuous pressure-shadows.

Mineral Differentiation

When rates of pressure-solution and growth on a crystal are not mutually compensatory there will be a net gain or loss of material and solute will migrate from one part of the rock to another. This may be brought about by mean x gradients resulting from differences in $\bar{\sigma}_A$.

The source region, under high $\bar{\sigma}_A$, therefore undergoes volume reduction and becomes relatively enriched in insoluble matter (clay, mica) because of predominant pressure-solution and depletion in soluble matter (quartz, calcite), while the reverse conditions hold in the region receiving solute. An example of mineral differentiation is shown in Fig. 3 by the differences in concentration of dark insoluble clayey material.

I thank Professors W. S. Fyfe and J. G. Ramsay and Dr M. S. Patterson for reading the manuscript, and NERC for a research studentship.

Received March 23; revised November 1, 1971.

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PKJKP

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Recent overtone data suggest that the Earth's inner core is soft with a shear velocity (β_1) in the range 2 to 4 km s⁻¹. A search for PKJKP corresponding to shear velocities in this range reveals five events consistent with $\beta_1 = 2.95 \pm 0.1$ km s⁻¹.

THE Earth's outer core is generally considered to be a fluid, at least within the resolution of seismic methods. On the other hand, the inner core¹, with a radius of about 1,250 km, has been the object of some discussion among seismologists. There is extensive evidence both that the boundary of the inner core is sharp², and that the velocity of compressional waves increases at this boundary. In one typical model³ the P-wave velocity rises from roughly 10.3 km s⁻¹ to 11.2 km s⁻¹ across the boundary. Within the inner core the P-wave velocity is almost constant.

It has often been conjectured that the inner core is solid. But searches for a seismic phase PKJKP travelling through the inner core as a shear wave have been unsuccessful, because of the small size predicted for the phase and uncertainty as to where to look for it. Bullen⁴ conducted a search in the distance range from 130° to 155°, based on the assumption of continuity of the bulk modulus across the inner core boundary which predicted an inner core shear velocity (β_1) of between 4 and 6 km s⁻¹.

Evidence for a "soft" inner core in which β_1 is only 2 or 3 km s⁻¹ has recently been put forward on the basis of free oscillation observations. Derr⁵ proposed a β_1 of roughly 2.2 km s⁻¹ as a good fit to free oscillation data, but commented that values would remain "highly uncertain" until reliable overtone data were obtained. Dziewonski and Gilbert⁶ make a larger compilation of free oscillation data including many overtones for which the inner core is well sampled. In particular, observations of modes ${}_2S_2$, ${}_5S_2$ and ${}_7S_3$ require an inner core shear wave velocity (assumed uniform) of roughly 3.5 km s⁻¹. This conclusion, in which it appears for the first time that a solid inner core is necessary, is also reported by Dziewonski and Gilbert⁷. More recently these workers (personal communication) indicate that a mean shear wave velocity for the inner core of 3.0 km s⁻¹ gives an equally good fit to the overtone data.

We thus started a search for the phase PKJKP using inner core shear-wave velocities of 2 to 4 km s⁻¹ and in this article we report the discovery of the phase and an estimate for β_1 of 2.95 ± 0.1 km s⁻¹. We do not see any compelling evidence for any radial dependence of β_1 .

Observational Data

Calculations of the epicentral distances (Δ) at which PKJKP should be largest were made with a ray tracing programme, which

takes into account both geometric spreading and the transmission coefficient at the inner/outer core boundary. We conclude that PKJKP would be largest in amplitude at epicentral distances between 200° and 290°. This corresponds to signals that arrive on the back azimuth at stations between 70° and 160° from an earthquake. Travel time tables so generated indicate that the signal should arrive roughly 30 to 33 min after the event, so we searched a small collection of records of large earthquakes at such epicentral distances from the Large Aperture Seismic Array (LASA) for possible PKJKP phases. We were prevented from increasing our distance coverage by the relatively sparse seismicity at distances of greater than 125° from LASA. The only other predicted seismic phases in this time interval are PKKP, SKKP and PcPPKP, and we shall show shortly that these can easily be identified and removed from consideration.

An array appears essential to this study because individual traces are still noisy, with complicated scattered signals, 30 min after origin time. An array gives us a slowness, or $dT/d\Delta$ measurement, for the phase. This is of great use in verifying that energy shown on a seismogram at a particular time is indeed associated with the correct phase. The vespagram process⁸ was used in this analysis; a typical vespagram is shown in Fig. 1. A prominent peak identified as PKJKP is seen with a $dT/d\Delta$ of 2.0 s deg⁻¹. This was a deep event and on the same vespagram can be seen both the AB and GH branches of the phase pPKKP. They are easily discriminated from PKJKP. In all vespagram analyses we have taken care to avoid identification of side lobes of other phases as PKJKP. In Fig. 2 we show a collection of waveforms of PKJKP, as identified by vespagram, together with PKKP from the same event. Data about the earthquakes used are given in Table 1.

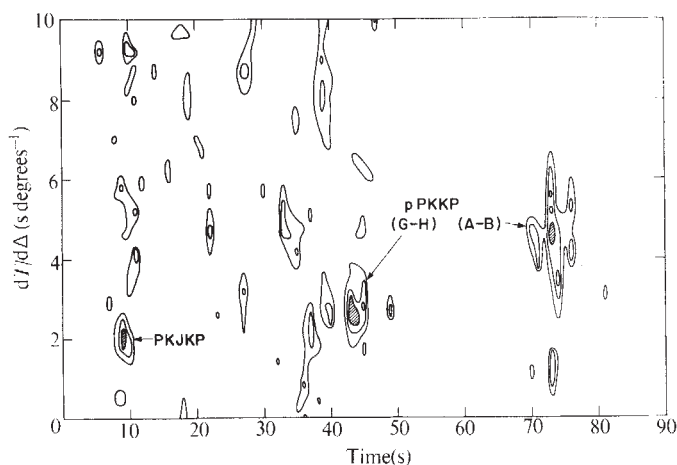


Fig. 1 A vespagram plot of short period seismic energy arriving at LASA as a function of time and slowness ($dT/d\Delta$) for the Fiji Island event. The contours are 2 dB apart; contouring only continues to 8 dB below the peak level (which is at PKJKP). The time scale begins at 17 : 52 : 50 GCT.

Table 1 Earthquakes Used in this Study

Date	Origin time (GCT)	Latitude	Longitude	Depth (km)	m_b	Region
June 17, 1967	5 : 00 : 12.0	53.36° S	26.83° W	136	6.3	S. Sandwich Is.
October 9, 1967	17 : 21 : 46.2	21.10° S	179.18° W	605	6.2	Fiji Is.
December 27, 1967	9 : 17 : 50.3	21.29° S	68.20° W	91	6.3	Chile-Bolivia
February 11, 1969	22 : 16 : 13.5	6.7° S	126.8° E	450	6.0	Banda Sea
July 18, 1969	5 : 24 : 48.0	38.4° N	119.4° E	33	6.1	NE China

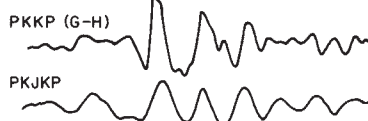
Table 2 Observed PKJKP Travel Times (Corrected to Surface Focus)

Date	Delta (deg)	Travel time
June 17, 1967	236.6	34 min 46.3 s
February 11, 1969	240.3	31 min 51.0 s
October 9, 1967	263.0	32 min 19.5 s
July 18, 1969	273.9	32 min 40.0 s
December 27, 1967	284.2	32 min 58.6 s

Each signal is the full array beam aimed in the direction of the maximum signal as indicated by the vespagram. Note the similarity of waveforms of PKJKP and PKKP at low frequency. We consider this to be strong confirmation of the reality of the phase. PKJKP is consistently depleted in high frequencies relative to PKKP, and we suppose this to be the result of attenuation within the inner core.

We have attempted to estimate the Q for shear waves in

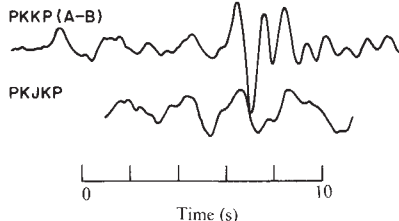
27 DEC 1967 $\Delta = 75.8^\circ$



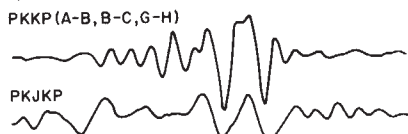
18 JULY 1969 $\Delta = 86.1^\circ$



9 OCT 1967 $\Delta = 97.0^\circ$



11 FEB 1969 $\Delta = 119.7^\circ$



17 JUNE 1967 $\Delta = 123.4^\circ$

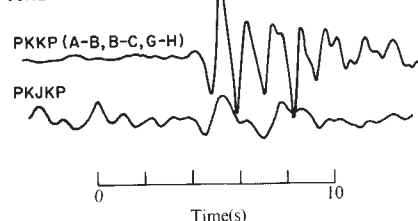


Fig. 2 Waveforms for each of the accepted PKJKP phases, with PKKP recordings (branch indicated) of the same event. The best beam is shown in each case.

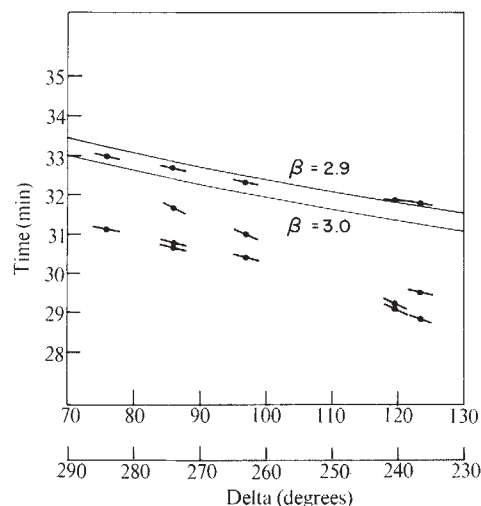


Fig. 3 The travel-time curve for observations of phases PKKP (lower points) and PKJKP with slopes ($dT/d\Delta$) indicated by vespagrams. Superimposed are theoretical travel-time curves for PKJKP with values for β_1 of 2.9 and 3.0 km s^{-1} .

the inner core by applying a variety of attenuation filters⁹ to the PKKP phases and choosing those which best reproduce the PKJKP waveform. Assuming that all the observed attenuation may be attributed to the inner core, we obtain a rough estimate for Q_β of between 500 and 1,000. Further work to refine this measurement better is currently under way.

Thirty-eight events of body wave magnitude greater than 6 were initially selected for study. Of these, nine appeared to be large enough for analysis by the VESPA process, and of these five had phases which could be identified unambiguously. A few other probable PKJKP phases were found, but side-lobe contamination could not be ruled out for these phases. None of the five PKJKP phases finally identified could have been detected on the output from any individual sensor. The observed travel times of PKJKP for these five events, corrected to a surface focus, are given in Table 2. In Fig. 3 these times are shown together with observed values of $dT/d\Delta$. PKKP is also included. The scatter in travel times for both phases is roughly 10 s, and is presumably caused by difficulty of picking the onset of the phase and uncertainties in the focal depth and origin time. Fig. 3 also shows the theoretical travel times for inner core shear velocities of 2.9 and 3.0 km s^{-1} . From these we infer that if the shear velocity were constant in the inner core it would have a value of $2.95 \pm 0.1 \text{ km s}^{-1}$.

We thank Freeman Gilbert for encouragement, and Clint Frasier for assistance. This work was sponsored by the Advanced Research Projects Agency of the Department of Defense.

Received December 13, 1971.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Extragalactic Origin of Low Energy Gamma Rays

THE existence of a finite flux of diffuse cosmic photons in the energy region of about 1 keV to about 1 MeV is well established¹⁻⁹. Furthermore, from observations on the isotropy of the radiation in the energy region of 1-100 keV, it is generally believed that a dominant fraction of the radiation in this energy band, if not all of it, is of extragalactic origin¹⁰⁻¹¹. On the other hand, at energies above ~ 200 keV, there is, as yet, no observational evidence for the isotropy or otherwise of the diffuse radiation, although some authors have advanced indirect arguments in favour of their extragalactic nature¹². More recently there have been attempts to interpret the diffuse cosmic gamma rays in the neighbourhood of 1 MeV as of galactic origin¹³. From a study of the directionality of the diffuse cosmic gamma rays between about 200 keV and 4 MeV, we present in this communication the first observational evidence of their extragalactic nature.

The gamma ray detector system used in the present experiment consisted of a $3'' \times 3''$ NaI(Tl) crystal and photomultiplier assembly, incorporated with a ^{241}Am "light-pulser" which provided a sharp inflight calibration line at an equivalent gamma ray energy of 2.68 MeV. A large plastic well with an average wall thickness of about 15 cm acted as an active collimator for low energy gamma rays; the FWHM value at 662 keV was about 55° . The plastic well, together with a thin plastic scintillator covering the front, provided a 4π shield for the detector against charged particles. The entire system was mounted on an orienting platform programmed in such a way that during the balloon ascent the telescope axis pointed up towards the zenith; on reaching ceiling altitude, it looked alternately towards and away from the galactic centre and Sco X-1 region in a direction towards the region of the galactic pole, for periods of 15 min. The balloon flight was conducted on December 2, 1970, from Hyderabad (geomagnetic latitude $\sim 8^\circ$ N); it reached a ceiling altitude of 6 g cm^{-2} .

Gamma rays which released in the detector crystal an energy in the range 0.25-4.2 MeV were analysed by a 256 channel pulse height analyser; the counting rates, suitably scaled down, were then recorded by an onboard camera. The 0.51 MeV positron annihilation line in the atmosphere and the 2.68 MeV inflight "light-pulser" line provided energy calibration during the entire flight period. In order to provide statistically significant data, the counting rates were grouped into five suitable energy bins. These data (recorded as a function of atmospheric depth, as the balloon ascended) were used to construct the growth curves for the five energy bins; typical growth curve plots are shown in Fig. 1. The presence of a cosmic component at atmospheric depths less than 10 g cm^{-2} is obvious from Fig. 1 because of the departure of these data points from the extrapolated straight line fitted to the points between about 10 and 50 g cm^{-2} . (Further arguments to substantiate the cosmic origin of the flux recorded at small atmospheric depths are given in ref. 3.) Thus the contribution due to cosmic photons can be evaluated from the growth curve and subsequently converted into cosmic photon flux for

a line source, using appropriate detector efficiency and atmospheric attenuation corrections. In Fig. 1 we also show the growth curve for the coincidence counting rate between the charged particle shield and the central detector; as is expected, this secondary component does not show the characteristic flattening of the growth curve up to the highest altitude reached. We found no significant difference between the count rates obtained at the ceiling altitude, when the telescope looked at the galactic centre, and those for a direction almost at right angles to the galactic plane. From this on-off method we can set upper limits to the photon flux from the galactic disk in the direction of the centre; these values are shown as upper limits in Fig. 2.

We believe that our observations favour an extragalactic origin of the diffuse soft gamma rays detected by our instrument during the time of ascent of the balloon. During the final stages of ascent of the balloon, when our instrument recorded excess counts due to cosmic photons, the galactic plane made an angle of 35° with the axis of the telescope. Because the FWHM at 662 keV is only 55° , our observations indicate that the finite flux of cosmic gamma rays, particularly in the two lower energy bins of 0.25 to 0.36 MeV and 0.36 to 0.63 MeV, is unlikely to be of galactic origin. If, however, we assume that the finite flux of cosmic photons seen is of galactic disk origin, we can calculate the expected photon flux from a direction along the galactic disk which, at the time of our observation, corresponds to a galactic longitude $l' \approx 55^\circ$. These flux values are shown in Fig. 2 by the solid line A (the corresponding flux value along the direction of the galactic centre, $l' = 0$, is likely to be somewhat higher than the line A of Fig. 2). But the upper limits to the photon flux from the galactic disk in the direction of the centre obtained from the on-off observations made in this experiment are already lower than the values indicated by line A in Fig. 2 by about an order of magnitude. These observations therefore demon-

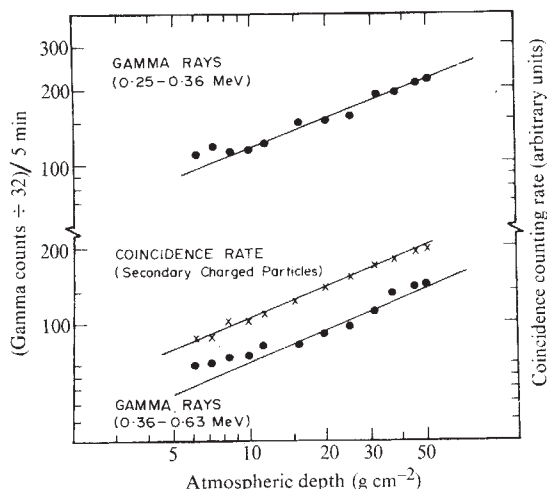


Fig. 1 Counting rate against vertical atmospheric depth for two gamma ray energy intervals and for the secondary charged particles measured in the present experiment. In all these cases, the straight line fit is made for data between 10 and 50 g cm^{-2} . The sizes of the data points (dots and crosses) determine the magnitude of the statistical errors. Departure from a straight line fit for the gamma ray points is due to the presence of a cosmic photon flux near the top of the atmosphere.

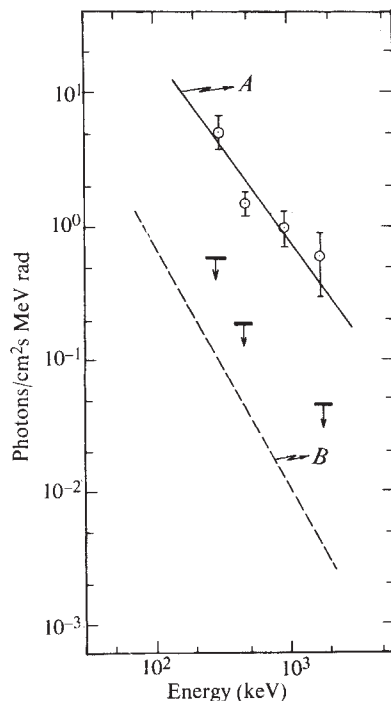


Fig. 2 Measurements and upper limits for the photons from the galactic disk. Curve A fitted to our data represents the large flux which would result if the finite flux of cosmic photons observed in the present experiment is attributed to the galactic disk; this contradicts the upper limits placed by our own measurement on the flux of photons from the galactic centre region. Curve B indicates the flux of X-rays to be expected from inverse Compton scattering of galactic electrons on the recently observed infrared photons. ∇ , Measured upper limits from galactic centre direction; $\bigcirc$, calculated flux on the galactic disk hypothesis.

in the Galaxy is 25 kpc, (ii) the thickness of the galactic disk is 2 kpc and (iii) the detector opening angle (FWHM) is 55° . Thus even this process, which can, if it exists on a galactic scale, dominate over all other presently known galactic processes for the emission of soft gamma rays, cannot account for curve A of Fig. 2.

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Received September 17, 1971; revised January 10, 1972.

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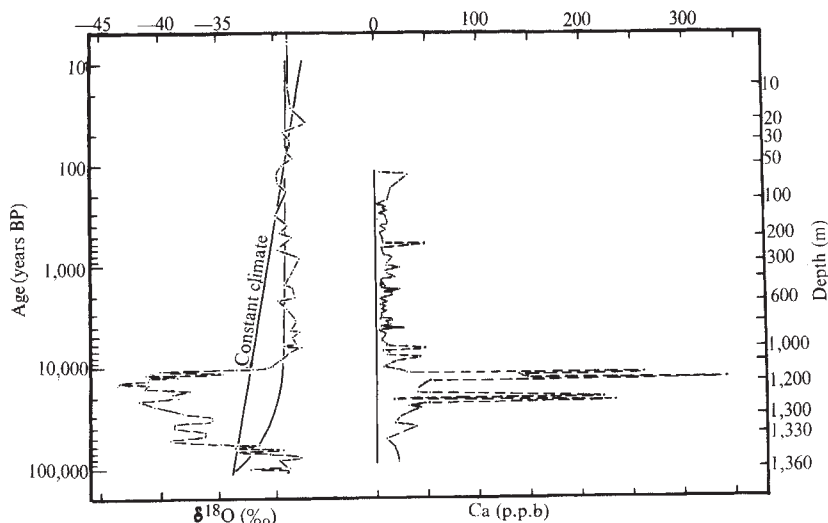
strate that the cosmic flux recorded by our instrument while the balloon was ascending is of extragalactic origin.

It would also be relevant here to compare the flux values from our measurements indicated in Fig. 2, with those expected from a process which can, in principle, yield the most liberal flux of gamma rays from the galactic disk. This process is the inverse Compton scattering of cosmic ray electrons in the Galaxy with the photons of the recently observed sub-millimetre radiation^{14,15}. It should be emphasized, however, that this argument is subject to the assumed existence of the sub-millimetre radiation on a galactic scale. The validity of this assumption is seriously disputed at present. Line B in Fig. 2 is this expected photon flux assuming (i) the radiating distance

Atmospheric Turbidity and Surface Temperature on the Polar Ice Sheets

DATA relevant to the causation of climatic change, particularly changes in mean annual temperature at the surface over intervals of from 10 to 10^5 yr, have been accumulating gradually in various laboratories engaged in studying the two long ice cores from Camp Century, Greenland, and "New" Byrd Station, Antarctica. This note consolidates these and other

Fig. 1 Camp Century, Greenland, $\delta^{18}\text{O}$ and calcium concentration profiles^{5,9}. Two hypothetical $\delta^{18}\text{O}$ profiles, assuming no climate change, are indicated by solid lines on the $\delta^{18}\text{O}$ profile. They represent two extreme cases in selection of flow lines for ice midway in the core.



data and interprets them in the framework of existing theory. It appears that millennial and longer variations in cloud-level temperature on the polar ice sheets have been caused by changing atmospheric turbidity over the past 10^5 yr.

The theoretical basis for a causal relationship between atmospheric turbidity and temperature has been developed by Rasool and Schneider¹, who showed that increased turbidity can reduce surface temperatures. Previously, many investigators had proposed such a relationship^{2,3}. Also, major volcanic eruptions have been followed by measurably cooler intervals⁴.

Measurements of oxygen isotope ratio ($\delta^{18}\text{O}$) have been made on samples of the Camp Century and Byrd cores^{5,6} revealing the existence of a cold interval roughly between 70,000 and 10,000 yr ago. Gow⁷ found volcanic ash layers in the Byrd core which are almost completely confined to that cold interval. Moreover, cation concentration data for both cores^{8,9} show that impurities and $\delta^{18}\text{O}$ are negatively correlated in both profiles.

Figs. 1 and 2 illustrate the calcium (Ca) concentration data and $\delta^{18}\text{O}$ values for the Camp Century and Byrd cores⁹. Even without any climate change, $\delta^{18}\text{O}$ would become more negative with depth because glaciers flow downhill; ice encountered in the core some distance beneath the drill site originated upglacier at an elevation higher (colder) than the drill site. The solid lines on the $\delta^{18}\text{O}$ profiles in Figs. 1 and 2 represent hypothetical profiles assuming no climate change. The flow models used to construct the hypothetical profiles require that ice at the bottom of the core originated at the present ice divide, and ice halfway to the bottom originated halfway between the drill site and the present ice divide¹⁰. The two hypothetical profiles at Camp Century represent two extreme cases in selection of flowlines and the midpoint.

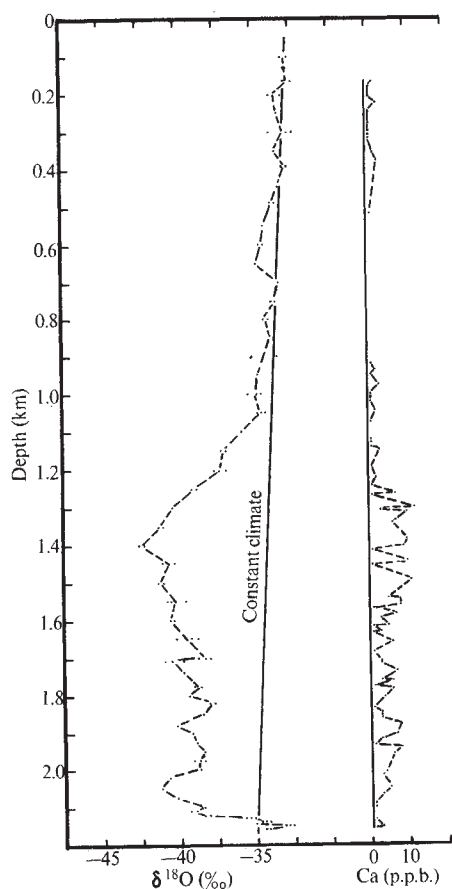


Fig. 2 "New" Byrd Station, Antarctica, $\delta^{18}\text{O}$ and calcium concentration profiles^{6,8}. The hypothetical $\delta^{18}\text{O}$ profile, assuming no climate change, is indicated by a solid line on the $\delta^{18}\text{O}$ profile.

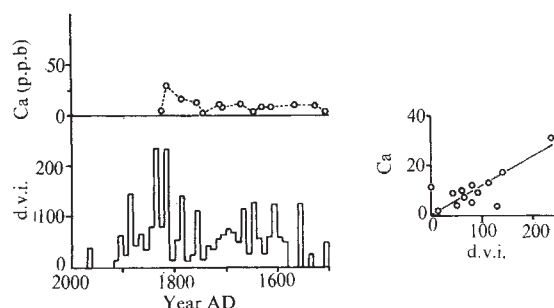


Fig. 3 Lamb's⁴ northern hemisphere volcanic dust veil index and calcium concentration in the Camp Century core⁹, since AD. 1500. The core chronology was determined from Dansgaard *et al.*⁵. The dust veil index (d.v.i.) has been normalized, by decade, to annual values.

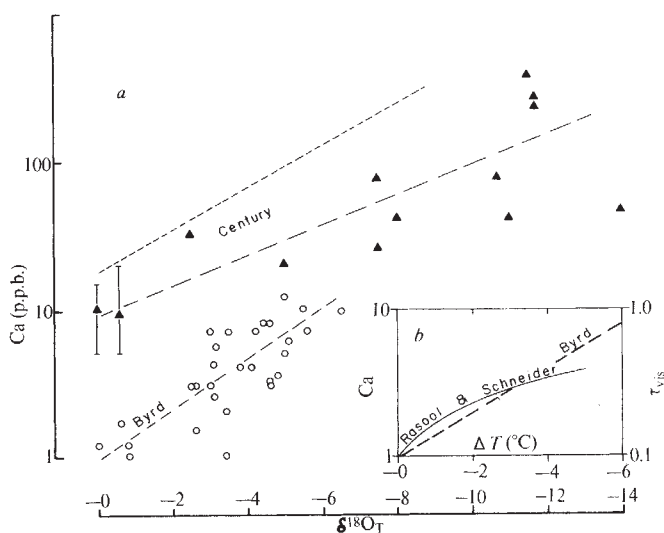


Fig. 4 *a*, Calcium concentration (p.p.b.) and oxygen isotope ratio difference ($\delta^{18}\text{O}_T$) from the hypothetical values at Camp Century and "New" Byrd Station. $\delta^{18}\text{O}_T$ is the amount of change of $\delta^{18}\text{O}$ which cannot be attributed to glacier flow in a constant climate situation, and is believed to be the amount of isotope ratio change due to variation in mean annual temperature at the drill site. The upper Camp Century line, for which points have not been plotted, is based on the straight line hypothetical profile of Fig. 1. *b*, Rasool and Schneider's¹ theoretical relationship between optical thickness of the atmosphere (for visible light) and surface temperature for a single scattering albedo of 0.9 is compared with palaeotemperature change induced by turbidity at "New" Byrd Station.

For the purposes of this communication the palaeotemperature change (ΔT) at the drill site is equal to $\delta^{18}\text{O}_T/0.9$, where $\delta^{18}\text{O}_T$ is the difference between the measured isotope ratio and the hypothetical isotope ratio. The proportionality constant (0.9 per mille $^{\circ}\text{C}^{-1}$) is the value measured by Picciotto *et al.*¹¹. It is apparent from inspection of the figures that the correction for flow improves the correlation of $\delta^{18}\text{O}$ with Ca concentration.

To establish a relationship between atmospheric turbidity and temperature, it is necessary to relate Ca concentration to dust concentration in the ice and to dust concentration in the air. Murozumi *et al.*¹² found that 90% of the Ca in Greenland snow was associated with silicate dust, with the remainder of the Ca ascribed to sea salt aerosol. Vittori and Prodi¹³ have shown that in laboratory experiments, where ice crystal scavenging was the predominant mechanism for removal of dust, dust concentration in the precipitation is directly proportional to the dust concentration in the air. Moreover, Lamb's⁴ dust veil index for volcanic eruptions since AD 1500 compares very well with the Ca concentration data⁹ for the Camp Century core (Fig. 3).

Correlation diagrams, comparing $\delta^{18}\text{O}_T$ and Ca concentra-

tion, are presented in Fig. 4a. The scatter is quite small considering that there is from ± 5 to ± 10 m uncertainty in matching cation and oxygen samples. The curves suggest an exponential relationship between $\delta^{18}\text{O}_T$ and Ca. This supports the supposition that increased turbidity is responsible for temperature decrease. An alternative explanation, that polar temperature decrease could produce the needed ten to twenty times increase in turbidity through strengthened meridional transport to the polar regions, seems unlikely.

Rasool and Schneider's attenuation curve (for a single scattering albedo of 0.9) is plotted in Fig. 4b along with the Byrd core Ca concentration— ΔT relationship. Optical thickness (τ_{vis}) is proportional to atmospheric dust concentration, hence Ca concentration, and the alignment of these values on the vertical axis in Fig. 4b is fixed by their present values. The agreement in slope is quite good, but it is also interesting to see that the ice core evidence suggests a decreasing cooling rate at higher values of turbidity, unlike the theoretical predictions of Rasool and Schneider.

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Palaeogravity from the Compaction of Fine-grained Sediments

It has been suggested that the gravitational coupling constant may have been weakening¹⁻⁴ or the Earth's radius increasing⁵ over geological time. Either effect would cause a decrease in gravity (g) at the Earth's surface. The rate of any such decrease is likely to be roughly 1 part in 10^9 per year (ref. 6). But Faytel'son^{7,8} has reported g varying throughout large areas of the Soviet Union, with mean rates over a decade of roughly ± 1 part in 10^7 per year. This rules out the possibility of direct measurements of any steady decrease, and to obtain mean rates of change over millions of years a gravity sensitive geological system is required. The effect of a declining gravity force on the compaction of sediments may provide a suitable indicator⁹.

If gravity in the past had been higher than today it is conceivable that some fine-grained sedimentary deposits would be overconsolidated, that is, compacted more by the smaller sedimentary column above them in the past than by the larger one existing today. Compaction is relatively rapid and largely irreversible, so the requisite evidence would be preserved.

Fig. 1 shows the effects of three different gravity models on the compaction of a particular volume of sediment undergoing progressive burial. Model A assumes gravity to be constant. The weight of overburden p , which increases with time, can be computed from Terzaghi's equation:

$$p = g(\gamma_s z - \gamma_w h)$$

The average density of the overburden is γ_s and that of the pore water is γ_w . Overburden thickness is z and pore water head h . To a close approximation $z = h$ and $\gamma_w = 1$ so that

$$p = g(\gamma_s - 1)z$$

Because the rate of sedimentation is assumed constant, z is proportional to t , the time elapsed since deposition of the volume of sediment in question. Thus

$$p \propto gt$$

Model B assumes a hypothetical decline in g according to the equation

$$g = \frac{t_0}{t} g_0$$

When sedimentation begins ($t=0$), g is infinite. It declines to g_0 (the present value of gravity) as t approaches t_0 (the present epoch). If gravity varied in this way (gt being constant) p would always have its present value, p_0 .

The third model, C, is more realistic in assuming that g declines linearly with time. Until time T gravity would be too low to achieve an overburden pressure p_0 , but afterwards it would exceed that needed and the sediment would be overconsolidated. The critical rate of decline in g , above which the sediment at the base of the sequence would be overconsolidated, is given by the gradient of the tangent to the hyperbola at $t=t_0$, that is, $-g_0/t_0$. The longer the period of uniform sedimentation the smaller the rate of decline in g which is detectable. The critical rate of decline is equivalent to a halving of gravity during sedimentation. Perceptible overconsolidation would, however, probably only ensue if gravity had declined rather faster—say, to one-third of its original value. Fig. 1 also shows the effects of various rates of gravity decline on the course of consolidation.

It is possible to discover whether or not sediments from a given sequence are overconsolidated by subjecting "undisturbed" samples to compression in the laboratory. A recent review of the consolidation characteristics of a wide range of sedimentary sequences (deposited over the last 50,000 years or so) shows them all to be normally consolidated¹⁰. These sediments were deposited in environments ranging from shallow marine (water 200 to 400 m deep) to intertidal and estuarine.

It is difficult to apply this method to sediments deposited over longer periods of time. Consolidation tests are usually undertaken in connexion with foundation studies and few results are available for depths greater than a few tens of metres. The thickest (and oldest) sedimentary sequences for which consolidation test data are published are sands, clays and silts of Plio-Pleistocene age in California¹¹. Results are shown in Fig. 2. Boring 24/26-36 A2 is significantly overconsolidated even at quite shallow depths. At the bottom of the hole, however, where the sediments are 7.5 m.y. old, overconsolidation is hardly perceptible. Boring 19/17-22 J1, J2 is underconsolidated at depth. Part of the sediment load is, presumably, still supported by the pore water—the process of consolidation has not yet been completed. Thus, although the older sediments are not overconsolidated in either of these holes, the anomalous nature of the sedimentation-compression curves means that little significance can be attached to the result.

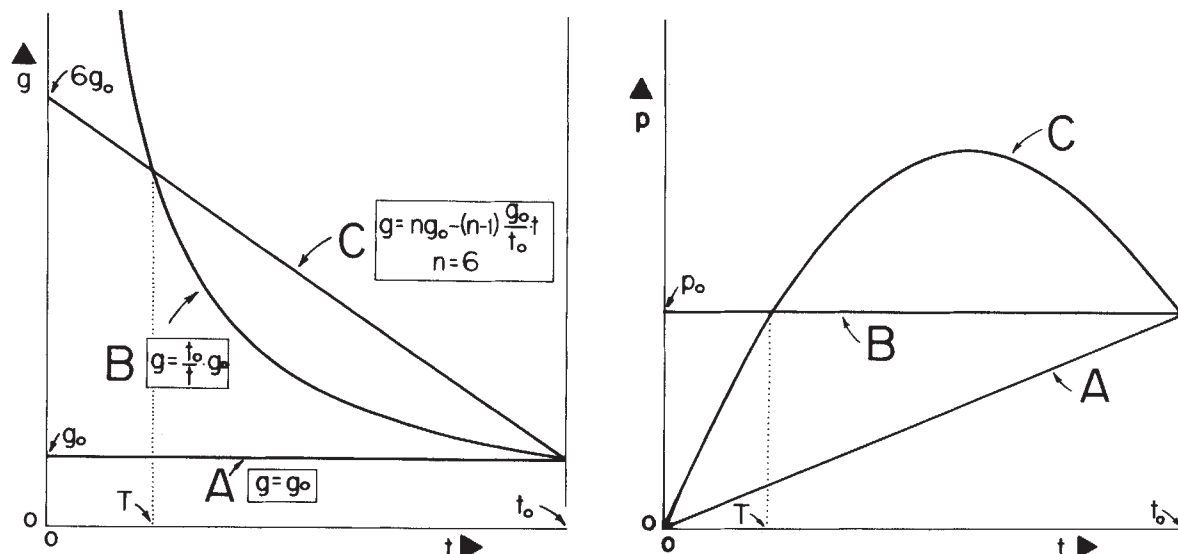


Fig. 1 Alternative gravity models (left) and their effect on overburden pressure (right) during uniform sedimentation. Time t is measured from the initiation of sedimentation in a given sequence.

The deep oceans provide sedimentary sequences spanning long periods. There, sedimentation was continuous and relatively uniform in rate compared with shallower water deposits. Consolidation tests might therefore be expected to yield ideal data. In fact, they are disappointing. Overconsolidation is the rule for sediments deposited during the past 50,000 years^{12,13}. This contradicts the evidence from contemporaneous shallow water deposits. Even the top few centimetres of some deep ocean sediments are substantially overconsolidated¹⁴. These were deposited during the past 10,000 yr, and gravity would need to diminish at the rate of

about 0.3 mgal per year in order to cause such overconsolidation. There is no evidence from instrumental observations over the past 150 years of such a high mean rate of decline in gravity. Whether this overconsolidation is the result of interparticle bonding or of the very low rate of sedimentation¹⁵ it renders present results from deep ocean sediments useless for the purpose in hand.

A somewhat different technique which might be used to measure gravity in the past is to examine the degree of overconsolidation of sediments which were once deeply buried beneath a sedimentary pile of known thickness, but have now been exhumed by erosion. An example is provided by the London Clay now exposed at the surface in the London basin. Undisturbed samples from three localities have been subjected to compression tests. Results are given in Table 1.

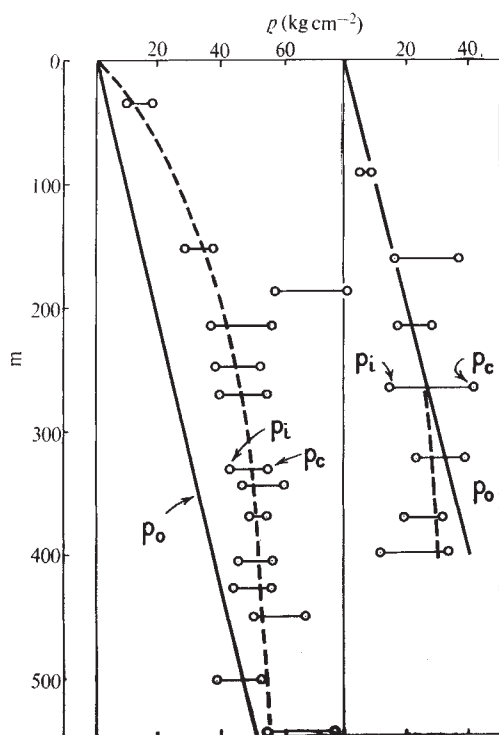


Fig. 2 Actual and expected consolidation data plotted against depth for each of two deep wells through fine-grained sediments in California. Left, boring 24/26-36 A2; right, boring 19/17-22 J1, J2. The expected effective overburden pressure p_0 was computed from density and depth measurements. The actual pressure sustained by the samples falls between two values p_1 and p_2 which were obtained from consolidation test results contained in ref. 11. Technical details are given in ref. 10.

Table 1 Results of Compression Tests in the London Basin

	Ashford Common (near Staines)	Waterloo Bridge	Bradwell (20 km NE of Southend)
Overburden pressure from tests kg cm^{-2}	42 ± 5	35 ± 5	~ 16
Equivalent overburden thickness metres	330-420	310-410	100-200
Overburden from geological evidence metres	> 205	> 220	> 76
References	17, 18	18, 19	20, 21

The sediments above the London Clay attain their greatest thickness in the Windsor area only a few kilometres west of Ashford Common. It seems nearly certain that the London Clay at Ashford, and probably also at Waterloo Bridge, had to support at least this thickness of overburden. The youngest sediments in the London basin are Eocene in age. Erosion, and hence unloading, probably began in Upper Oligocene-Lower Miocene times¹⁶, roughly 26 m.y. BP.

It is clear from Table 1 (see refs. 17 to 21) that gravity could not have had more than twice its present strength at the time unloading began, or the London Clay would be compacted more by the existing overburden than is observed. Alternatively, an unchanged gravity force would imply the former existence of an additional 100-150 m of sediment younger than any now known in the London basin. This is quite feasible geologically because many hundreds of metres of Oligocene sediments are known from Hampshire and the Paris basin. They form the upward continuation of a succession essentially identical to that seen in the London basin.

So the compaction of clay technique eliminates any decline in g greater than 6 parts in 10^5 per year. The more powerful exhumation technique eliminates any decline greater than 4 parts in 10^8 per year over the past 26 m.y. An accurately known overburden thickness would increase the precision of the method by almost an order of magnitude and allow detection of a weaker gravity field than at present.

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Global Distribution of Eulerian Poles

THE root of present day rigid plate tectonics is Euler's fixed axis theorem, whereby the displacement of any fraction of the total surface of a sphere which does not carry every point on it into itself is a rigid rotation about an axis which passes through the centre of the sphere (Eulerian axis), and pierces the surface of the sphere in two fixed antipodal points (Eulerian poles).

In another context, Euler's theorem is the initial premise in Weyl's elegant geometrical determination of the five finite groups of proper rotations in three dimensional space¹.

Le Pichon² located five centres of rotation (otherwise Eulerian poles) at 70° S 118° E (SP), 58° N 37° W (NA), 78° N 102° E (A), 53° N 47° W (NP), and 26° N 21° E (IO), corresponding to the opening of the South Pacific, the Atlantic, the Arctic, the North Pacific and Indian Oceans.

Three of these (NA/NP, IO) and their antipodes NA'/NP', IO', lie on or near the great circle GC1 of Fig. 1. The other two (A, SP) and their antipodes A', SP', together with the pairs NA/NP and NA'/NP', define a second great circle GC2. Furthermore, A/SP' and A'/SP lie with Le Pichon's Indian Ocean pole IO on or near a third great circle GC3.

There results a topological network of eight spherical triangles, with the pole pairs NA/NP, NA'/NP', A/SP', A'/SP, plus the solitary Indian Ocean Pole IO and its antipode IO', lying tolerably close to the intersections of their boundaries.

In terms of group theory, the symmetry of the six points of intersection of our three great circles parallels that of the class of six four-poles characteristic of the octahedral group¹.

The parallel is, however, imperfect, in that not only are the six four-poles of the octahedral group single, but the incipient double-pole symmetry of the network displayed in Fig. 1 is flawed in the absence of a companion to the Indian Ocean pole.

In the search for such a companion, an analysis of the pattern of transform faults revealed by the bathymetry of Lake Tanganyika and Lake Nyasa in the East African Rift Valley³, to which my attention was drawn by Professor H. W. Wellman, disclosed it at about 21° N 20° E, as the centre of rotation of the "East African Subplate" envisaged by Dietz and Holden⁴.

A graphical check rates the chance against the possibility

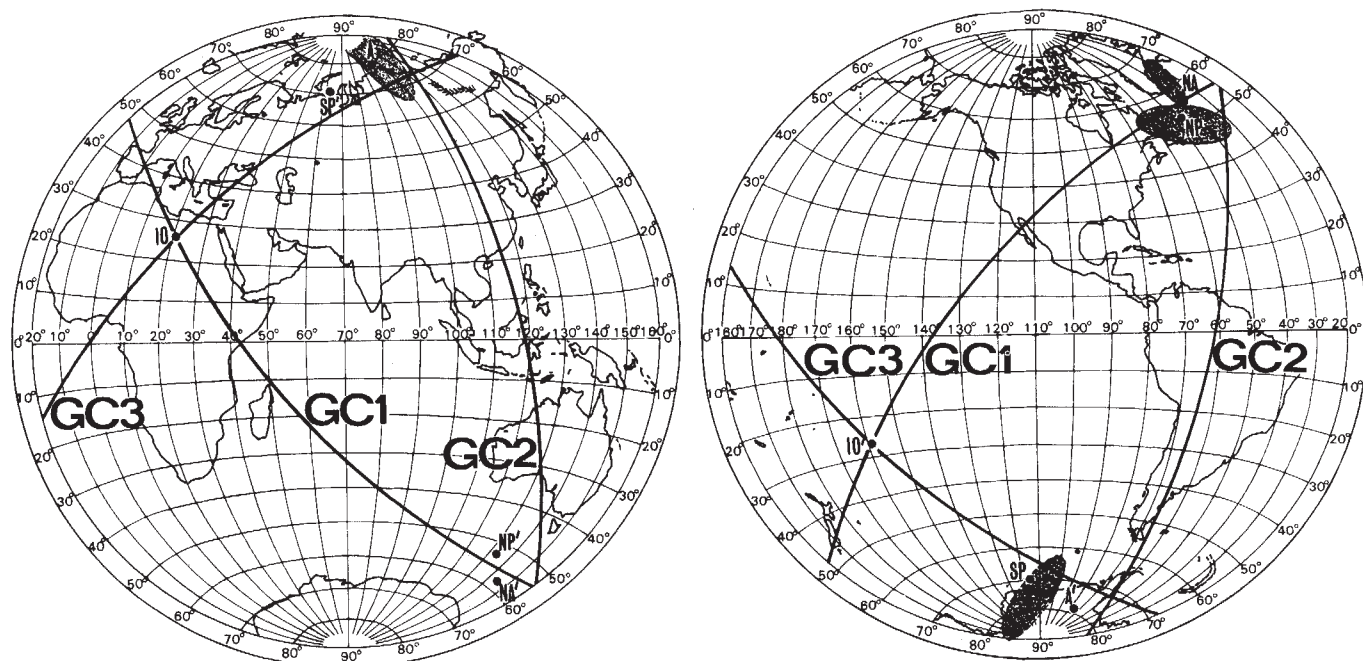


Fig. 1 Six Eulerian poles, five as located by Le Pichon², the sixth disclosed as the centre of rotation of the "East African Subplate" by Dietz and Holden⁴, lie in pairs on or near the three great circles GC1, GC2 and GC3. The Scotia Arc and the antipodal Verhoyansk Mountains are seen to lie alongside the intersections of the great circles GC2 and GC3.

that the final pattern of six pairs of Eulerian poles so arrived at might be a random distribution at more than 20 to 1.

We remark that the Scotia Arc and its antipode in the Verhoyansk Mountains lie alongside the intersections of the great circles GC2 and GC3.

It scarcely needs emphasizing that none of the above, any more than the rigid plate tectonics from which it derives, is other than phenomenological—the how of it, in fact. The why is another matter.

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Compound Classification by Computer Treatment of Low Resolution Mass Spectra—Application to Geochemical and Environmental Problems

THE large number of mass spectra that are generated by gas chromatographic-mass spectrometric (GC-MS) examination of lipid fractions are being processed more and more by on-line digital computers¹⁻⁴.

Computers have already been applied to the problem of sorting and interpreting the spectra⁵⁻⁹. The approaches taken include the use of artificial intelligence¹⁰, learning machines¹¹, matching unknown spectrum to a file or library¹²⁻¹⁹ and of classification of mixtures²⁰⁻²⁴. Unfortunately, existing procedures commonly require extensive programs and considerable computer time.

We here describe a development of computer analysis of low resolution GC-MS data, which provides a preliminary classification of an unknown spectrum as a listing of candidate classes of compounds²⁵. The analyst may then select those spectra which merit detailed interpretation and study. This capacity to sort, arrange, and effectively display in order of importance is of great value in dealing with complex mixtures of compounds. The method is essentially the file or library search approach²¹. COMSOC (Classification of Mass Spectra on Computers) operates by converting an incoming unknown mass spectrum into a simplified "key word" which is then compared with each of the sixty-five or so key words held in its reference file (the "correlation set"). The computer then prints out the best matched reference key words as a listing of the chemical classes they represent. For convenience in dealing with homologous series of compounds, chemical class is defined as $R-(CH_2)_n-H$ where R is constant and differing values of n (0, 1, 2, ...) do not result in changing fragmentation patterns.

The compact key word used by COMSOC is the ion-series spectrum²⁵ or reduced mass spectrum²⁶. All m/e values are arranged in a rectangular array of fourteen columns numerically equivalent to the values for hydrocarbon ions C_nH_{2n-11} to C_nH_{2n+2} . The percentage contribution of each of the fourteen ion series to the total ion count is given by

$$\sum_{35} \left[\frac{(m/e \ 30 + m) + 14n}{\sum_{35}} \right] \times 100 \quad \begin{matrix} n = 0, 1, 2, \dots \\ m = 1-14 \end{matrix}$$

For a given mass spectrum, the intensity values for all m/e values with mass differences of 14 are listed in the appropriate column. Each column is then summed to provide the ion-

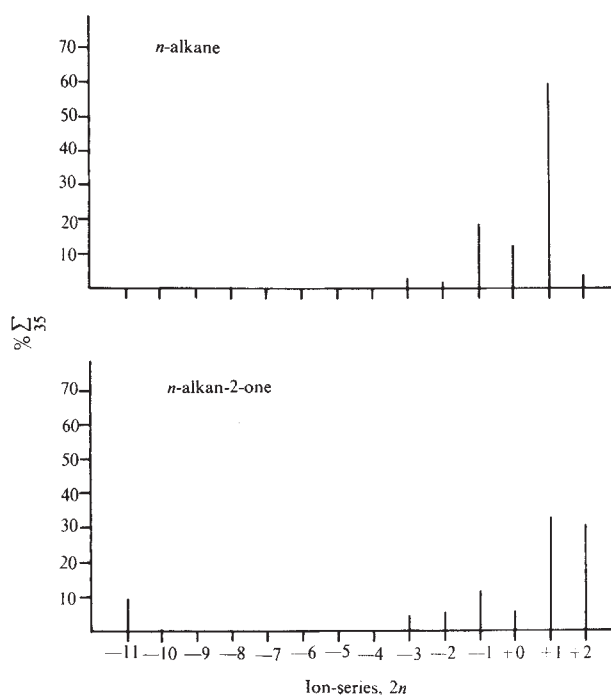


Fig. 1 Ion series spectra for the compound classes n -alkane, $CH_3(CH_2)_nH$ ($R=CH_3$, $n=8-36$), and n -alkan-2-one, $CH_3CO(CH_2)_nCH_3$ ($R=CH_3CO-$, $n=8-27$).

series spectrum for that compound. This fourteen column arrangement of total ionization percentage against ion series is particularly appropriate to the classes of compound where the CH_2 group (mass 14) is the basic repeating unit of the homologous series. The ion series spectrum is essentially constant for a particular class, $R(CH_2)_nH$, being relatively independent of the value of n especially above the first few members of a series. This constancy is well known for certain classes of hydrocarbons such as n -alkanes, n -alkenes and simple benzenoid hydrocarbons²⁵. Indeed, ion series spectra have been used for many years as a means of assessing the contribution of these hydrocarbons to petroleum and other materials. More recently, on-line computer displays have been used in the preliminary examination of lunar samples^{27,28} and advocated by Crawford and Morrison²⁶. An extension of the ion series approach to the methyl esters of straight-chain and monomethyl-substituted fatty acids was initiated in 1967 by Pettersson and Ryhage^{13,29}. Recently, Biemann *et al.*¹⁹ have used ion series classification as a preliminary procedure prior to a full file search requiring the matching of the complete mass spectrum of an unknown compound.

In our work we have used an ICL 4-75 to process both our spectra and spectra selected from over 9,000 held on magnetic tapes supplied by the Mass Spectrometry Data Centre (MSDC), Aldermaston. The library spectra were originally recorded in several laboratories, on different instruments and in a wide variety of operating conditions. The spectra for many of the sixty-five classes of compound, however, are remarkably constant for members of the single class concerned, especially for molecules larger than C_{10} . Accordingly, we have assembled a correlation set of sixty-five or so ion series spectra (for m/e values > 35) which summarizes much of the information in the main library and is stored in the computer as about 1,000 16-bit words. Further, the ion series in the set vary sufficiently from one class to another such that they perform the essential role of providing the discrimination between classes (Fig. 1). Classes at present represented in the correlation set include a variety of normal, branched and cyclic alkanes, alkenes, benzenoid compounds, polynuclear aromatics, ethers, aldehydes, ketones, esters and amines.

CLASSIFIER

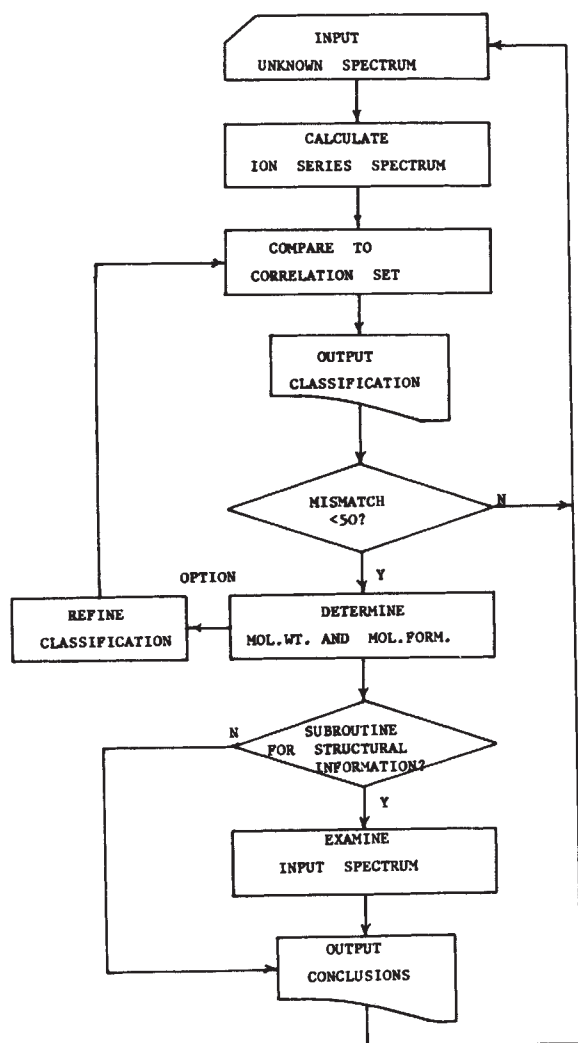


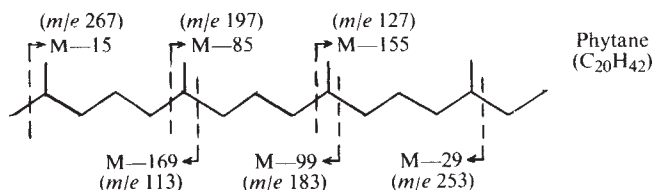
Fig. 2 Flow chart of the COMSOC program, illustrating the basic steps in the classification of mass spectra. N, No; Y, yes. The program is chiefly in Fortran, run on an ICL 4-75.

The operation of COMSOC is outlined in Fig. 2. The Mismatch term is the sum of the absolute differences between the ion-series summation values of the unknown and the reference class.

$$\text{Mismatch} = \sum_{n+1}^{14} \text{absolute value (unknown series}_n - \text{correlation set series}_n)$$

It ranges from 0 to 200 and provides a numerical indication of the matching of an ion series spectrum and a particular ion series spectrum held in the correlation set. Listings of classes giving a low value (< 50, indicating a good match) are obtained and a search is then carried out to locate a molecular ion or an ion in a particular ion series appropriate to the class assigned. These routines give molecular formulae. Special subroutines keyed to this information then search for characteristic "anomalies" in certain ion series, generally leading to the print-out of best matches for a small number of classes. Thus, a branched alkane subroutine is used to discriminate among the various classes of geochemically important acyclic alkanes (*n*, *iso*, *anteiso*) and isoprenoid. This routine examines the distribution of ions in the $2n+1$ series (m/e 57, 71, 85 . . .), which for *n*-alkanes decreases monotonically with increasing mass: anomalies or discontinuities are sought for which are characteristic of the classes, for example,

M-15 and M-43 for *iso*-alkanes and M-29 and M-57 for *anteiso*-alkanes. Table 1 gives the computer output obtained when the mass spectrum of phytane was processed by COMSOC. The characteristic pattern of intensity anomalies separated by 70 mass units (M-29, M-99, M-169 and M-15, M-85, M-155) results in final classification as an isoprenoid alkane.



We have experimented with COMSOC as an aid to the GCMS analysis of mixtures, using the manual processing of raw mass spectral data presently available to us. These included the organic content of the effluent from a carbon-black factory, and the total alkane fractions isolated from two oil shales of Eocene age ($\sim 50 \times 10^6$ yr) and from a contemporary Norwegian lichen.

Table 1 Summary of Computer Output for Mass Spectrum of Phytane submitted as Unknown

	Mismatch	Class
Phytane	5.7	Isoprenoid alkane
	9.9	Highly branched alkane
	18.9	Iso-alkane
	20.0	<i>n</i> -Alkane
	20.6	<i>anteiso</i> -Alkane
Conclusion: acyclic alkane		
Molecular weight is 282		
Molecular formula is C ₂₀ H ₄₂		
Anomaly at mass 113 (M-169)		
Anomaly at mass 127 (M-155)		
Anomaly at mass 183 (M-99)		
Anomaly at mass 197 (M-85)		
Anomaly at mass 253 (M-29)		
Anomaly at mass 267 (M-15)		
Conclusion: probable isoprenoid alkane		

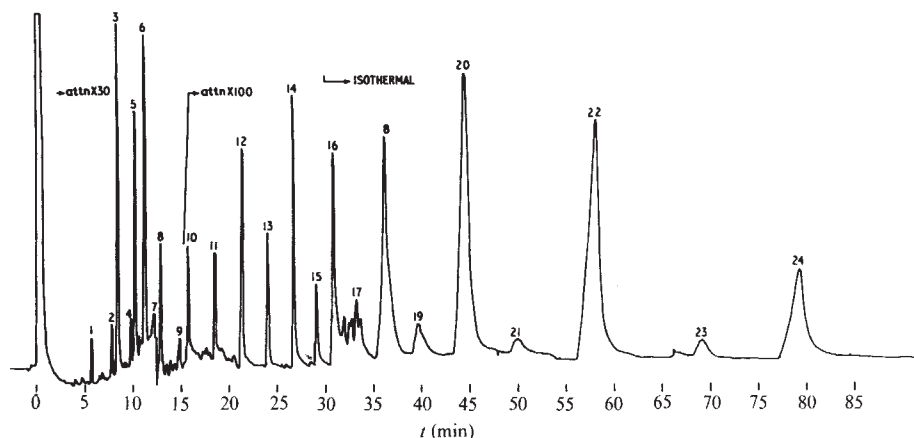
The total ion current monitor trace observed when an aliquot of the alkane fraction obtained from the lichen *Siphula ceratites* was subjected to GCMS analysis is shown in Fig. 3. Twenty-four spectra were recorded. The computer output from COMSOC is summarized in Table 2. The major components of the mixture are *n*-alkanes. They form a homologous series from *n*-C₁₅ to *n*-C₃₃ (with the exception of *n*-C₃₂) and show a predominance of odd/even carbon numbers, but several other components are present in significant amounts.

The preliminary characterization of this mixture was carried out in less than 30 s of computer central processor time. Most peaks were classified unambiguously and the molecular weights and formulae derived on the basis of the classification served to identify these compounds.

The COMSOC, as at present developed, provides a preliminary classification of an unknown spectrum as a listing of candidate classes. Further development will include extension of the number of classes in the correlation set, more sophisticated treatment by subroutines and inclusion of other chemical, chromatographic and spectroscopic data. These data, for example gas chromatographic Kovats indices for specified phases, would be used to verify or improve initial classification and structure identification. Judicious choice of derivatization techniques and computing procedures should permit the major analytical steps to be carried out in the computer rather than at the bench.

Compound classification based on ion series spectra seems to be a useful method for preliminary characterization of complex mixtures analysed by GCMS techniques. Modifica-

Fig. 3 Total ion current monitor trace for a Varian MAT CH-7 GCMS run of the total alkane fraction of the lichen, *Siphula ceratites*. Column conditions: 1/16 inch $\times$ 10 foot stainless steel, 3% OV-17 on 100–120 mesh 'Chromosorb W'. Flow rate 5 ml./min helium. Temperature programmed 150°–270° C at 4°/min. Mass spectrometer conditions were ionizing voltage 70 eV, 100 μ A emission current. Accelerating voltage was 3 kV. Spectra were scanned linearly from m/e 10–500 in 5 s. The total alkane fraction was obtained by preparative thin-layer chromatography (TLC) on AgNO₃-impregnated silica gel plates.



tions in progress which will encompass operation on small laboratory computers, should considerably extend its utility, but this simple and inexpensive method, which defines or severely restricts the compound class to which an unknown spectrum belongs, will facilitate the use of both the artificial intelligence¹⁰ and library matching^{12–19} approaches to the interpretation of mass spectra. For example, a reorganization of the available large libraries of mass spectra along the lines of compound class would enable library search techniques to be exercised on a much smaller data file. This reorganization would also permit more facile development of alternative or additional correlation sets, which might be in number arrays other than the fourteen ion series used herein.

Finally, the matching and classifying procedures based on a correlation set as used here should have direct application in other areas of science. Chemotaxonomy, for example, requires the ordering of species according to patterns of compound abundances. Similar treatment of normal and abnormal patterns of biolipid abundances would be of value in biochemical and biomedical studies.

We thank SRC, NERC and NASA for financial support and

NASA for a fellowship (to D. H. S.). We thank the Office of Scientific and Technical Information for a grant; Dr T. Bruun (Trondheim) for the lichen fraction and Mr N. A. B. Gray for assistance.

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Received August 16; revised October 22, 1971.

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Table 2 Classification Results for Spectra recorded in the GCMS Analysis of Lichen Alkanes (Fig. 3)

Peak	Final classification data		Mol. formula	Mismatch
	Class	Mol. weight		
1	n-Alkane	212	C ₁₅ H ₃₂	6.5
2	n-Alkane	226	C ₁₆ H ₃₄	2.7
3	Tricyclic alkane	220	C ₁₆ H ₂₈	36.1
4	Isoprenoid alkane	268	C ₁₉ H ₄₀	29.9
5	n-Alkane	240	C ₁₇ H ₃₆	6.9
6	Acyclic alkane	254	C ₁₈ H ₃₈	*
7	Unclassified	—	—	—
8	n-Alkane	254	C ₁₈ H ₃₈	13.6
9	Unclassified	—	—	—
10	n-Alkane	268	C ₁₉ H ₄₀	5.4
11	n-Alkane	282	C ₂₀ H ₄₂	4.9
12	n-Alkane	296	C ₂₁ H ₄₄	7.0
13	n-Alkane	310	C ₂₂ H ₄₆	10.3
14	n-Alkane	324	C ₂₃ H ₄₈	9.4
15	n-Alkane	338	C ₂₄ H ₅₀	8.4
16	n-Alkane	352	C ₂₅ H ₅₂	18.0
17	n-Alkane	366	C ₂₆ H ₅₄	5.1
18	n-Alkane	380	C ₂₇ H ₅₆	7.7
19	n-Alkane	394	C ₂₈ H ₅₈	4.4
20	n-Alkane	408	C ₂₉ H ₆₀	7.9
21	n-Alkane	422	C ₃₀ H ₆₂	8.0
22	n-Alkane	436	C ₃₁ H ₆₄	10.1
23	Triterpane-IV	—	—	25.3
24	n-Alkane	464	C ₃₃ H ₆₈	7.0

* Mismatch = 14.8 for anteiso-alkane class.

Peak 3 is only poorly matched with class of perhydroanthracenes and perhydrophenanthrenes. Peaks 4, 6, manual inspection suggestive of pristane and a mixture of 7- and 8-methylheptadecane³², respectively. Peak 23, lack of appropriate molecular ions indicates computer classification as triterpane sub-class IV is incorrect and that the present library is inadequate.

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Direct Methods in Neutron Crystallography: Structure of L-Proline Monohydrate by Symbolic Addition and Tangent Refinement

"DIRECT METHODS" for the solution of crystal structures lead directly to the phases of the structure factors from the distribution of the intensities of Bragg reflexions. Most of these methods depend on the assumption that the scattering density is everywhere positive, an unassailable assumption for the electron density associated with X-ray scattering. In neutron diffraction the scattering density for hydrogen atoms is negative, so that it becomes questionable whether the same techniques are applicable. One possible answer to the question has been suggested by Karle in the squared structure approach¹, and the structure of glycolic acid has been solved by Ellison and Levy² following Karle's suggestion. Sikka³ has suggested that the conventional symbolic addition method⁴ may be used in neutron crystallography when the scattering by hydrogen is less than 1/3 of the total scattering:

$$\sum_H b_H^2 / \sum_{\text{all atoms}} b_i^2 < \frac{1}{3}$$

where b_i is the neutron scattering length. The structure of trichloroacetic acid, $\text{Cl}_3\text{CCO}_2\text{H}$, was solved by direct methods from neutron diffraction data by Jönsson *et al.* (unpublished work). This is a centrosymmetric structure, and the amount of hydrogen scattering is small, so that a successful structure solution by application of the Σ_2 relationship⁴ was not surprising.

Acentric crystal structures generally offer more difficulties in solution. We report here the first solution of a complex acentric organic crystal structure from the application of direct methods to neutron diffraction data. The experimental data for L-proline monohydrate were obtained as part of the Brookhaven program which has as its goal the precision refinement of structures of all the principal naturally-occurring amino-acids. L-Proline monohydrate has the formula $\text{C}_5\text{H}_{11}\text{O}_3\text{N}$, and the percentage of hydrogen scattering (27%) approaches the limit suggested by Sikka: the method "... cannot be applied when the contribution of the negative atoms to the total scattering is above 30%. The upper limit for the number of atoms in the unit cell is around 100".

Preliminary crystallographic data on L-proline monohydrate have been reported by Sasisekharan⁵ who gave the space group as C2 (monoclinic). The following are least-squares refined cell dimensions from the neutron diffraction data ($\lambda = 1.013(1)$ Å): $a = 20.56(3)$ Å, $b = 6.308(8)$ Å, $c = 5.176(7)$ Å, $\beta = 93.64(2)^\circ$, $Z = 4$.

Normalized structure factors (E s) were calculated for the full set of unique reflexions and gave good distribution statistics. A total of 210 reflexions with a smooth distribution of E values between 1.20 and 2.88 were used to generate 1,440 Σ_2 relationships. One reflexion with a higher E value (3.7) was not included. A set of five starting phases was selected automatically by the computer program MULTAN⁶: two origin defining reflexions, plus three reflexions to which were assigned in turn the thirty-two non-enantiomorphous combinations of phases obtained with the values $+45^\circ$, $+135^\circ$, $+225^\circ$, $+315^\circ$. The Germain, Main and Woolfson weighted tangent refinement method⁶ was then used to calculate thirty-two sets of refined phases for all 210 reflexions. The associated figures of merit for these sets ranged from 1.51 to 0.97.

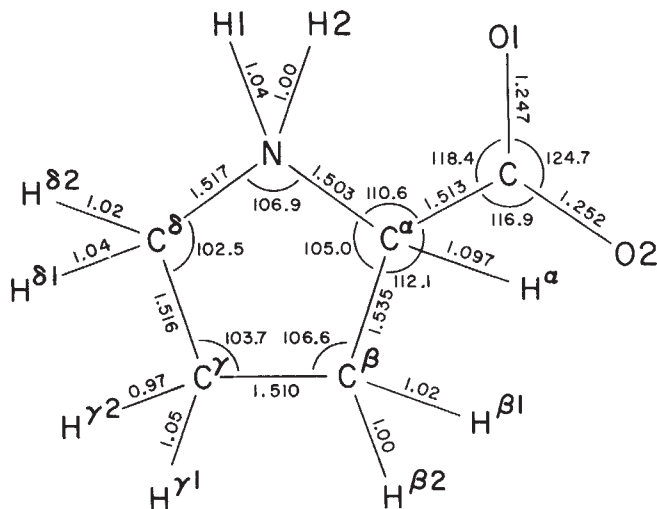


Fig. 1 Structure of L-proline monohydrate.

E -maps were calculated for the thirty-two solutions. The five sets with the highest figure of merit (1.51–1.50) were found to be trivial: many of the phases were close to 0 or 180° . The next two sets with $M = 1.316$ and $M = 1.307$ revealed the correct structure: both E -maps showed six well-resolved peaks above the background level, the carboxyl group and $\text{N}-\text{C}^\alpha-\text{C}^\beta$ (Fig. 1). The second one also showed a seventh, weaker peak corresponding to the water oxygen atom. The two solutions differed essentially by a shift of 0.1 in the polar coordinate γ . The first six atoms were included in a structure factor calculation, which gave $R = [\Sigma ||F_o| - |F_c||] / \Sigma |F_o| = 0.37$ for the 537 strongest reflexions.

Iteration of Fourier syntheses and structure factor calculations quickly revealed all the missing atoms in the structure.

A full-matrix least-squares refinement is in progress. Some of the hydrogen atoms show very high anisotropic temperature factors, a phenomenon not unusual for amino-acids, and we are considering the use of higher order cumulants⁷ to obtain a more appropriate description of the thermal motion. At the present stage, the agreement between squared observed and calculated structure amplitudes is $R = [\Sigma ||F_o|^2 - |F_c|^2|] / \Sigma |F_o|^2 = 0.077$ for 664 reflexions with $I_o \geq 3\sigma(I_o)$.

The bond distances and angles in the proline molecule are given in Fig. 1. The estimated standard deviations are 0.005 Å for heavy atom distances, 0.3° for angles and 0.02 Å for distances involving hydrogen. The C–H and N–H distances will have large corrections for thermal motion. A complete description of the molecular and crystal structure, including the extensive hydrogen bonding, will be published when the generalized thermal motion refinement is complete.

We conclude that Sikka was not too optimistic in his predictions and that direct methods can probably be applied to neutron diffraction data for almost any crystal to which they are applicable to X-ray diffraction data.

This work was performed under the auspices of the US Atomic Energy Commission. We thank the Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture, Belgium, and Statens Naturvidenskabelige Forskningsråd, Copenhagen, for travel grants to J. J. V. and M. S. L., who is on leave from Kemisk Institut, Aarhus Universitet. T. F. K. is a postdoctoral fellow of the US National Institutes of Health.

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Received October 8, 1971.

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BIOLOGICAL SCIENCES

In vitro Transcription of *Escherichia coli* Ribosomal RNA Genes

THE ribosomal cistrons of *Escherichia coli* comprise only 0.2 to 0.4% of the genome and yet in exponentially growing cultures 40% of the instantaneous rate of RNA synthesis can be ribosomal RNA^{1,2}. These genes are under stringent control, which means that they are not expressed in wild type (*rel*⁺) cultures starved for amino-acids³. Travers *et al.*^{4,5} postulated that a protein factor, called ψ r, is a positive regulator of the rRNA genes, responsible for both their preferential synthesis and their turn-off during amino-acid starvation. This appealing hypothesis rests on their report that purified RNA polymerase synthesizes no detectable rRNA from *E. coli* DNA. They concluded that addition of ψ r (a protein factor found either in crude extracts of uninfected *E. coli* or as a com-

Table 1 Summary of Experiments using *in vitro* RNA to Compete with ³²P-rRNA

Enzymes	Amount of synthesis relative to E	% of rRNA
(1) E σ (holoenzyme)	1.0	12
(2) E (core)	0.72	1
(3) E σ + ρ	0.5	8
(4) E σ + ψ r from uninfected cells	1.9	9
(5) E σ + Q β III and IV	1.5	11
(6) E σ + 0.8 mM ppGpp	0.48	8
(7) E σ + ψ r uninfected + 0.5 mM ppGpp	1.25	8

All reactions had the final concentration of salts described in Fig. 3 and except for the reaction of line 2 contained 70 μ g/ml. of *E. coli* DNA and 20 μ g/ml. of RNA polymerase holoenzyme and were incubated for 30 min at 37° C. Unless otherwise indicated the RNA was extracted, precipitated and resuspended in a final volume of 400 μ l. of 2 $\times$ SSC and the total amount of RNA synthesized determined as described in Fig. 3. The amount of rRNA was also determined as in Fig. 3. In all cases parallel hybridizations containing known amounts of rRNA were run as standards. The amount of total synthesis and the amounts of rRNA synthesized relative to the holoenzyme reaction were determined by parallel reactions and hybridizations. All TCA precipitations and hybridizations were done in duplicate, and all experiments were repeated at least three times. Lines 1 and 2 are from Fig. 1. Line 3: a saturating amount of highly purified ρ factor (as determined by a depression assay) was added to one of two 1.0 ml. reactions before the DNA was added. It was found that 4.31 μ g of RNA was synthesized in the absence of ρ factor and 2.10 μ g in its presence. Line 4: 100 μ l. of the peak fraction of the stimulation activity (prepared on a 0.5 M KCl glycerol gradient from an extract of uninfected *E. coli* strain PR-7 as described by Travers *et al.*⁴) was added to one of two 0.5 ml. reactions. Then 3.22 μ g of RNA was synthesized in the presence of the stimulation activity and 1.52 μ g in its absence. Line 5 is from the reaction described in Fig. 4. Line 6: one of two 500 μ l. reactions was 0.8 mM in guanosine tetraphosphate prepared by the method of Cashel⁶. Here, 1.24 μ g of RNA was synthesized in the presence of ppGpp and 2.53 μ g in its absence. Line 7: the reactions are similar to those described in line 4 except that two reactions containing the gradient purified stimulation activity were run, one of which was 0.5 mM in guanosine tetraphosphate. Here, 1.53 μ g of RNA was synthesized in the absence of the stimulation activity, 3.02 μ g synthesized in its presence, and 1.70 μ g in the presence of both the stimulation activity and the guanosine tetraphosphate.

Table 2 Hybridization Competition of *in vitro* ³H-RNA by rRNA

	Amount of synthesis relative to E	% rRNA minimum	Estimated %
(1) E σ	1.0	7.2	10
(2) E σ + ψ r	2.2	5.6	8
(3) E σ + ppGpp	0.48	5.4	8
(4) E σ + ρ	0.51	5.4	8.5
(5) E σ + MSII	0.45	7.3	10

The reactions were similar to those described in Fig. 6. All were done in parallel with a reaction identical to that described in Fig. 6 to determine the relative amount of RNA synthesized. Reactions were stopped with 2 ml. of 2 $\times$ SSC and extracted with 0.1 ml. of 2 $\times$ SSC saturated phenol and adjusted to 10⁵ c.p.m. (³H)/ml. by dilution with 2 $\times$ SSC. ³²P-labelled rRNA was added to bring the ³²P c.p.m. to 10⁴ counts/ml. Then 0.1 ml. of mixture was TCA precipitated as described in Fig. 3. Aliquots of 100 ml. of this mixture were added to 0.1 ml. of 0.05 M NaPO₄, pH 6.7, buffer, containing 0.7 μ g of separated strands of *E. coli* DNA enriched for rRNA and to 0.1 ml. of the DNA and 0.4 μ g of rRNA added to one set. The hybridizations were incubated for 3 h at 67° C, RNAase digested, collected, washed and counted as described in Fig. 3. For all samples the hybridization efficiency of ³²P-rRNA was measured by the percentage of ³²P precipitable counts retained by the filters after hybridization in the presence of DNA. In the absence of competitor this was, however, near 70% and the hybridization efficiency of ³H-RNA near 10%. The competition for binding rRNA, as judged by the percentage of ³²P-rRNA retained by filters after the hybridization in the presence of 0.4 μ g of rRNA, was in all cases about 95%. The minimal amount of rRNA is determined as the percentage of TCA precipitable ³H counts which are actually competed away by addition of rRNA. The estimated percentage is calculated on the assumption that ³H-rRNA hybridizes with the same efficiency as does ³²P-rRNA. All TCA precipitation and hybridizations are done in duplicate. Each line in the table has been repeated at least twice. Line 1 is from Fig. 6. Line 2: 20 μ l. of the stimulation activity from uninfected cells purified on a 0.5 M glycerol gradient as described by Travers *et al.*⁴ was added to a 0.1 ml. reaction and incubated for 15 min at 37° C. Line 3: similar to line 1 with 0.5 mM ppGpp added. Line 4: similar to line 1 but with a saturating amount of ρ factor added. Line 5: similar to line 1 but 0.8 mM MSII purified as described by Cashel *et al.*⁶. MSII is a guanosine pentaphosphate compound accumulated in *rel*⁺ but not *rel*⁻ strains of *E. coli* starved for amino-acids.

ponent of Q β replicase) to the *in vitro* reaction specifically stimulates rRNA synthesis so that it accounts for 30% of the transcript. They also reported that ψ r does not stimulate rRNA synthesis in the presence of guanosine tetraphosphate (a phosphorylated nucleoside accumulated in stringent cells starved for amino-acids)^{6,7}. Of the *in vitro* product, 10% is rRNA in the presence or absence of ψ r.

I have confirmed the observation that preparations of Q β replicase subunits III and IV and the 3S region of a crude extract of uninfected cells stimulate transcription by holoenzyme from *E. coli* DNA^{4,5}. This stimulation, however, is not specific for rRNA. Something between 7 and 14% of the RNA transcribed *in vitro* by highly purified RNA polymerase holoenzyme (containing the σ factor) from highly purified *E. coli* DNA is rRNA. The ψ r factors are not required for this high level of synthesis and do not increase the percentage of the transcript which is rRNA. Guanosine tetraphosphate (ppGpp) does not decrease the percentage of rRNA in the RNA product in the presence or absence of the ψ r factors.

The amount of rRNA in the transcript as determined by using *in vitro* RNA to compete with ³²P-labelled rRNA for complementary sites on denatured *E. coli* DNA is shown in Table 1. Table 2 tabulates the results of a second assay in which rRNA is used to compete with *in vitro* ³H-RNA (to which ³²P-rRNA is added as an internal control) for sites on single stranded *E. coli* DNA, enriched for the rRNA cistrons.

Both assays show that about 10% of the transcript is rRNA, regardless of whether or not the ψ r factors are present in the *in vitro* reaction. The reaction conditions are similar to those that have been used before^{4,5}. The order of addition of the DNA and the RNA polymerase to reactions containing the ψ r factors does not affect the result. In other experiments the percentage of the transcript which is rRNA is constant within

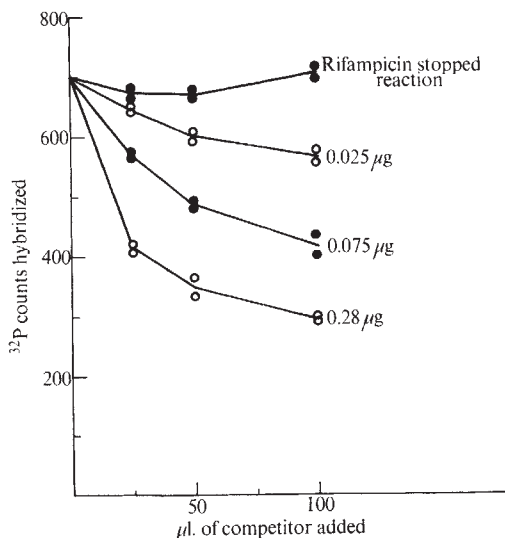


Fig. 1 Hybridization competition with known amount of rRNA. 5 µg/ml. of rifampicin (Le Petite) was added to a 2 ml. standard reaction mixture before the addition of 40 µg of RNA polymerase holoenzyme. The reaction was incubated for 30 min at 37° C and divided into four 0.5 ml. aliquots. No rRNA was added to the first; 0.025 µg rRNA to the second; 0.075 µg to the third; and 0.28 µg to the fourth. The reactions were then prepared for and used in hybridization competition reactions as described in Fig. 3 except the pellet was resuspended in 400 µl. of 2×SSC.

the range of 0.1–0.2 M KCl in the presence or absence of the stimulation activity from uninfected cells (Table 3).

Fourteen different DNA preparations extracted and purified in several different ways^{7,8} have been used. With the exception of two, about 10% of the transcript was rRNA, whether the DNA was extracted from exponentially growing or stationary cultures. I detected no rRNA synthesis from the same DNA (now after 1.5 yr) used by Travers, Kamen and Schleif⁴ (in the experiment from which they concluded that less than 0.2% of the RNA synthesized is rRNA). This preparation of DNA and another (purchased from Sigma Biochemicals), which gave the anomalously low yield of 2% rRNA, probably have extensive single stranded regions which cause aberrant synthesis. A large fraction of the RNA synthesized on these templates is resistant to ribonuclease digestion and is retained by nitrocellulose filters. (Dr A. Travers informs me that at the time he used the DNA, 5% of the RNA transcript was resistant to digestion by RNAase.)

Table 3

KCl concentration	ψr	Amount of synthesis relative to E at 0.1 M KCl	Minimum %	Estimated %
0.10 M	—	1.0	7.1	10.2
	+	1.8	6.9	10.0
0.15 M	—	0.75	5.8	8.5
	+	1.6	6.8	9.5
0.175 M	—	0.49	6.4	9.0
	+	1.4	6.8	9.0
0.20 M	—	0.44	5.8	8.5
	+	1.3	6.5	9.0

Reactions were similar to those described in Fig. 6 except that the concentration of KCl was varied from 0.1 M KCl to 0.2 M KCl. One set of reactions contained the 20 µl. of the stimulation activity purified on a 0.5 M KCl glycerol gradient as described by Travers *et al.*^{4,5}. The reactions were incubated for 15 min at 37° C. The RNA was extracted and hybridized as described in Table 1. The relative level of synthesis is normalized to the 0.1 M KCl reaction in the absence of the stimulation activity. The reactions were stopped with 1 ml. of 2×SSC and diluted until they contained 10⁵ c.p.m. (³H)/ml. Before hybridization, 1.0×10⁴ c.p.m./ml. of ³²P-rRNA (0.1 mCi/µmol in 2×SSC) was added to each tube.

The difference in the results is not attributable to the RNA polymerase preparations. Six different preparations of holoenzyme, including the batch used for most of the original ψr experiments⁴, yield 10% rRNA in the transcript. None of the preparations contain traces of 35,000 or 45,000 molecular weight proteins, as judged by SDS gel electrophoresis, which would correspond to subunits III and IV of Qβ replicase. RNA polymerase purified by the methods of Burgess⁹ and Berg, Barrett and Chamberlin¹⁰ can be used interchangeably.

I found the assay in which rRNA is used to compete with ³H *in vitro* RNA for sites on denatured DNA^{4,5} to be unreliable. Uncontrollable variations in this assay may be due to the fact that only about 10% of the ³H-RNA is retained by the filters after hybridization and to the problems inherent in looking for small differences under such circumstances.

Pettijohn *et al.*¹¹ reported that they could not detect rRNA in the *in vitro* product using holoenzyme. A value of 10% rRNA borders on the limit of the sensitivity of their assay, and consequently they may have been unable to detect it.

The assays used here are sensitive to small amounts of ribosomal RNA and do not rely on small differences between large numbers. The assay described in Fig. 1 can detect a concentration of 0.02 µg/ml. of rRNA. It specifically measures the concentration of rRNA in the *in vitro* product. Increasing the concentration of rRNA in the reaction increases the ability of the extract of the reaction to compete with ³²P-rRNA as expected (Fig. 1). R17 and f2 RNA do not compete with the ³²P-rRNA even when present at ten times the concentration of rRNA needed for exhaustive competition. Similarly RNA synthesized by holoenzyme from T4, T7 and calf thymus DNA templates does not compete either (Fig. 2). Fig. 3 demonstrates that the *in vitro* RNA product of holoenzyme does compete with ³²P-rRNA but the product of core enzyme¹² does not. The concentration of rRNA in the holoenzyme

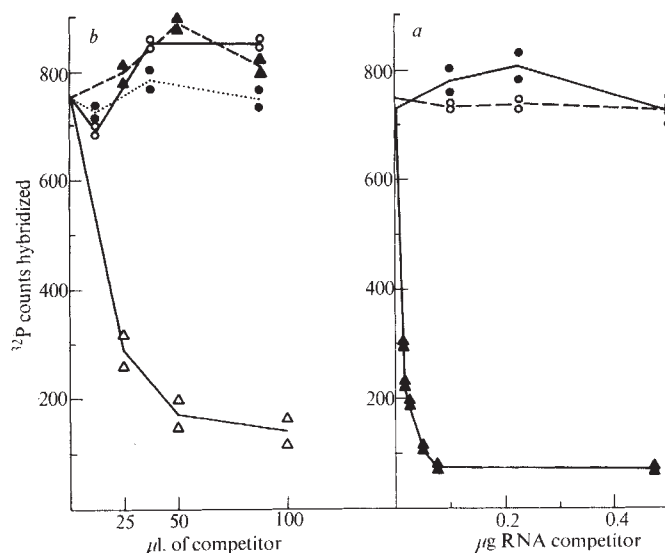


Fig. 2 Specificity of the hybridization competition. *a*, 2 µg of purified R17 RNA (○), purified f₂ RNA (●) and purified rRNA (▲) were added to three 400 µl. of 2×SSC and the solutions used in the hybridization competition reactions described in Fig. 3. *b*, 400 µl. standard reactions, containing (instead of *E. coli* DNA) 40 µg/ml. T4 DNA or calf thymus DNA, and 20 µg/ml. RNA polymerase holoenzyme, were incubated for 30 min at 37° C. The reactions were stopped and phenol extracted as described in Fig. 3. A 400 µl. reaction containing 70 µg/ml. of *E. coli* DNA and 20 µg/ml. RNA polymerase as described in Fig. 3 was run in parallel. The phenol phase was pipetted away and the concentration of RNA in the aqueous phase determined by TCA precipitating 10 µl. aliquots as described in Fig. 3. The concentration of the RNAs in all the aqueous phases was adjusted by dilution with 2×SSC to 2 µg/ml. and used in the rRNA competition hybridization as described in Fig. 3. Δ, *E. coli* DNA; ▲, T4 DNA; ●, λ DNA; ○, T7 DNA.

product is determined by comparing the competition curve with that of a reaction mixture containing a known amount of rRNA. Assuming that rRNA sequences synthesized *in vitro*

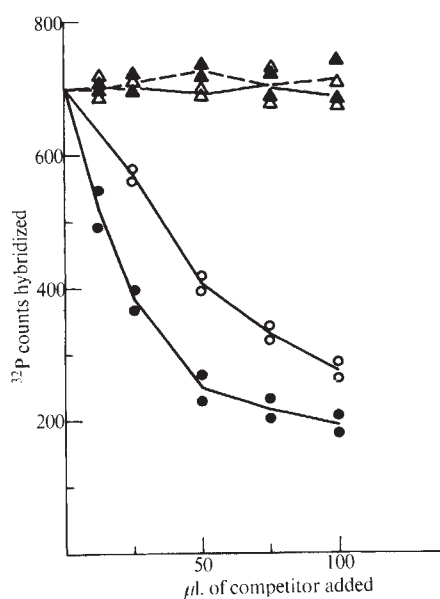


Fig. 3 Competition of ^{32}P -rRNA by ^3H -UTP labelled RNA *in vitro*. Four 1 ml. reactions were 0.02 M Tris (pH 7.9); 0.01 M KCl; 0.01 M magnesium acetate; 0.0001 M dithiothreitol; 0.2 mM ATP, GTP, CTP (PL Biochemicals); 0.05 mM UTP, 100 $\mu\text{Ci}/\mu\text{mol}$ (Schwartz Biochemical) contained 70 mg of *E. coli* DNA each. 5 μg of rifampicin was added to two of the reactions before 20 μg of RNA polymerase holoenzyme was added; 20 μg of RNA polymerase holoenzyme was added to a second reaction and 20 μg of core RNA polymerase (prepared by eluting the holoenzyme from phosphocellulose column as described by Burgess *et al.*¹²) to the third. Ribosomal RNA (0.2 μg) was added to the reaction containing rifampicin. After incubation for 30 min at 37° C, 10 μl . aliquots of the reactions were removed, dried on 'Millipore' filters and counted in a toluene-omni-fluor scintillation fluid. Another 10 μl . aliquot was removed from each reaction and the nucleic acids precipitated in the presence of 20 μg of BSA with 2 ml. of 6% trichloroacetic acid containing 0.01 M sodium pyrophosphate; this determines the total amount of acid-precipitable material. In the presence of rifampicin, no ^3H -UTP was incorporated into acid-precipitable material. The reactions were stopped by adding 1 ml. of 2 $\times$ SSC (0.3 M NaCl, 0.03 M Na citrate) and extracted once with 1 ml. of 2 $\times$ SSC saturated phenol. The nucleic acids in the aqueous supernatant were precipitated with 4 ml. of 95% ethanol for 2 h at 20° C and collected by centrifugation at 10,000g for 15 min. The precipitates were resuspended in 0.7 ml. of 2 $\times$ SSC and 10 μl . of each removed and TCA precipitated as described above. Recovery of acid-precipitable material was 98%. The total amount of RNA synthesized (4.2 μg by holoenzyme at 3.2 μg by core enzyme) was calculated from the percentage of the total ^3H -UTP in each reaction incorporated into acid precipitable material. Increasing amounts of the resuspended pellet were added to 100 μl . of 2 $\times$ SSC containing 0.45 μg of the denatured *E. coli* DNA and 0.0005 μg of a mixture of purified 16S and 23S labelled rRNA of specific activity 0.2 mCi/ μmol . The final volume of each hybridization reaction was adjusted with 2 $\times$ SSC to 200 μl . The reactions were incubated at 67° C for 3.5 h, diluted with 750 μl . of 2 $\times$ SSC containing 10 μg of RNAase A (Schwartz) and incubated for 30 min at 37° C. They were filtered through 'Schleicher and Schuell B-6' filters, rinsed with 40 ml. of 0.05 M Tris (pH 7.5), 0.5 M KCl, dried and counted. It was previously determined that 0.45 μg of DNA is within the linear region for hybridization of the labelled rRNA. With no competitor present 15% of the input ^{32}P -rRNA was retained by the filter. In the absence of denatured DNA, in the presence or absence of the maximum amount of the reaction mixture, less than 0.5% of the input ^{32}P -rRNA counts are retained. The RNA polymerase holoenzyme was purified from *E. coli* K-12 (purchased from grain processing) by the procedure of Berg *et al.*¹⁰. *E. coli* DNA was prepared from a saturated culture of RFS-1 (a strain obtained from R. Schleif) by the method of Miura⁷ followed by a pronase digestion as described by Marmur⁸. $\blacktriangle$, *In vitro* product of core enzyme; $\triangle$, rifampicin stopped reaction; $\circ$, rifampicin + 0.2 μg rRNA; $\bullet$, *in vitro* product of RNA holoenzyme.

compete with the same efficiency as does rRNA extracted from ribosomes, Fig. 3 demonstrates that $12\% \pm 2\%$ of the *in vitro* holoenzyme transcript is rRNA. Less than 1% of the RNA product of core enzyme is rRNA.

Fig. 4 illustrates a similar experiment in which Q β subunits III and IV¹³ (a gift of T. Blumenthal and T. Landers) were added to one reaction. The nucleic acids were extracted from the *in vitro* reactions and that from ψ r-stimulated reaction was diluted so that both extracts contained the same concentration of labelled RNA. The two RNA preparations show identical competition curves, which demonstrates that the percentage rRNA sequences are the same; about 10%, in both transcripts.

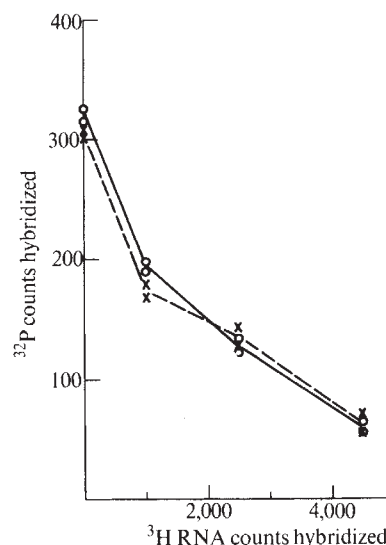


Fig. 4 The effect of Q β subunits III and IV on rRNA synthesis. The reactions are the same as those described in Fig. 3 except they are 400 μl . and contain 3.0 μg of DNA and 1.8 μg of RNA polymerase. A saturating amount (as judged by the stimulation assay) of purified subunits III and IV of Q β replicase was added to one of the reactions. The RNA was phenol extracted as described, the phenol phase removed and the RNA precipitated with 2 volumes 95% EtOH at -20° C, centrifuged for 30 min in a clinical centrifuge in the cold, resuspended in 400 μl . of 2 $\times$ SSC. The solutions (10 μl .) were TCA precipitated and counted as described, and 200 ml. of 2 $\times$ SSC added to reaction containing Q β subunits to equalize the RNA concentration of the two tubes. These solutions were then used in the hybridization reactions as described. It was found that 0.82 μg of RNA was synthesized in reaction 1 and 1.21 μg in reaction 2; 11% of RNA synthesized is rRNA in both cases. The curve is plotted as ^3H counts hybridized against ^{32}P -rRNA bound. $\times$ --- $\times$, $\text{E}\sigma + (\psi\text{Q}\beta\text{III} + \text{IV})$; $\circ$ — $\circ$, $\text{E}\sigma$ alone.

Addition of subunits III and IV of Q β replicase and ψ r from uninfected cells stimulates the transcription of total RNA from *E. coli* DNA by the holoenzyme (Table 1, lines 4 and 5). It is unlikely that this stimulation is due to random nicking of the template since Vogt¹⁵ has shown that limited treatment of various DNA templates with pancreatic DNAase I stimulates only transcription by the core enzyme and not by the holoenzyme. It is clear, however, that the stimulations observed here are not specific for rRNA (Table 1, lines 4 and 5, and Table 2, line 2).

Fig. 6 illustrates a typical assay using the other assay system. The DNA used in this procedure is prepared by eluting highly purified denatured *E. coli* DNA from a methylated albumin kieselguhr column with a salt gradient as described by Margulies *et al.*¹⁴ (a technique brought to my attention by J. Pero). DNA sequences complementary to rRNA elute at a higher salt concentration than does the bulk of the DNA (Fig. 5). Ribosomal RNA hybridizes efficiently to this DNA; routinely more than 70% of the ^{32}P -rRNA is retained by filters after hybridization whereas only about 10% of the *in vitro* product is retained. Purified R17 RNA added

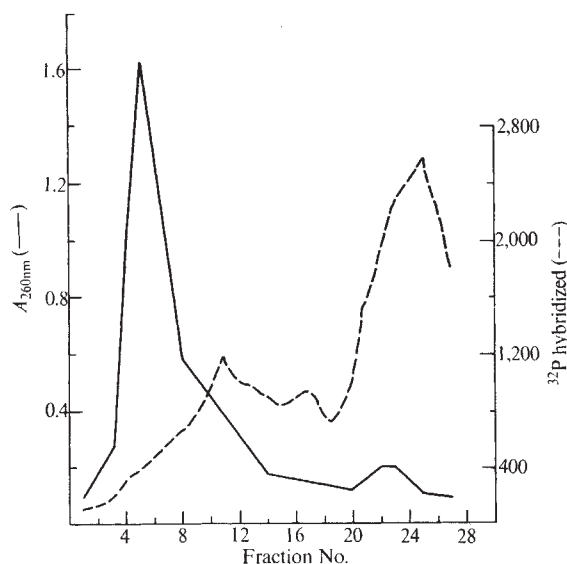


Fig. 5 Hybridization of a mixture of 16S and 23S rRNA to single stranded *E. coli* DNA eluted from an MAK column. *E. coli* DNA (10 mg) purified by the method of Miura⁷ (including two isopropanol precipitation steps) in 7 ml. of 0.1 M Tris (pH 7.9), 0.001 M EDTA, was denatured by adding 1.4 ml. of 1 M NaOH, diluted with 40 ml. of 0.05 M NaPO₄ (pH 6.7), 0.5 M KCl and dialysed overnight against three 1 l. changes of the phosphate buffer. The DNA was loaded on a column similar to the simplified MAK column described by Margulies *et al.*¹⁴. A thin layer of cellulose was layered over the sintered disk of a Kontes 25 mm×250 mm column and 10 g of 'Hyflosupercel' (Johns Manville), to which 4 mg of methylated albumin (Calbiochem) had been absorbed, was gently layered over the cellulose. This was topped with 1 g of 'Hyflosupercel' and the column washed with 200 ml. of the phosphate buffer. The DNA was loaded on the column in 50 ml. at room temperature at 0.5 M KCl in the phosphate buffer and the column washed with 100 ml. of the same buffer. Less than 1% of the A_{260} flowed through the column. The column was eluted with a 500 ml. linear salt gradient from 0.5 to 0.9 M KCl. The gradient was interrupted when the A_{260} reached 0.15 and eluted at that salt until the A_{260} began to drop and the salt gradient resumed at the interrupted concentration. Fractions of 8 ml. were collected. From every other tube containing appreciable A_{260} , a 100 μ l. sample was removed and added to 0.1 ml. of $2\times$ SSC containing 3.7×10^3 counts/ml. of ^{32}P -rRNA (0.1 mCi/ μ mol). The hybridization technique was the same as described in Fig. 3.

to a hybridization reaction at ten times the concentration of ribosomal RNA necessary for exhaustive competition of ^{32}P *in vivo* rRNA did not compete with either the ^{32}P -rRNA or the *in vitro* product.

The difference in the amount of 3H *in vitro* RNA retained by filters after hybridization in the presence or absence of saturating amounts of rRNA added as a competitor permits the calculation of the minimum amount of rRNA in the *in vitro* product. In Fig. 4, approximately 60% of the 3H -RNA retained by the filter after hybridization in the absence of competitor was competed away by an amount of rRNA sufficient to compete away 95% of the ^{32}P -rRNA in the same reaction mixture. In this experiment, 12% of the 3H *in vitro* RNA hybridized to the DNA which means that at least 7% of the *in vitro* product is rRNA. Assuming that the hybridization efficiency for sequences of rRNA synthesized *in vitro* is the same as the efficiency of authentic ^{32}P -rRNA (70%), about 10% of the *in vitro* product is rRNA, in good agreement with the estimate from the previous assay.

As measured by both assays the fraction of rRNA remains high in the RNA synthesized in the presence of a saturating amount of the termination factor ρ^{16} (a gift of E. Minkley) (Table 1, line 3, and Table 2, line 3). This factor decreases by 50% the total amount of RNA synthesized in a 30 min reaction and significantly shortens the length of the RNA chains as determined by gel electrophoresis. This experiment suggests

that there is no ρ -mediated termination signal between the promoters recognized *in vitro* and the ribosomal cistrons.

Cashel¹⁷ and Gallant *et al.*¹⁸ have postulated that guanosine tetraphosphate (ppGpp) is a direct inhibitor of rRNA synthesis *in vivo*, because they found that an early consequence of the *rel* gene function during amino-acid starvation is the rapid accumulation of ppGpp. Earlier reports indicated that ppGpp specifically inhibits the rRNA synthesized in an *in vitro* reaction containing ψ r. Contrary to these reports, I find that ppGpp does not decrease the fraction of rRNA when added to reactions with or without ψ r (Table 1, lines 6 and 7, and Table 2, line 3). However, ppGpp does decrease the total amount of RNA synthesized in both cases, as previously reported.

These experiments demonstrate that in the conditions used RNA polymerase has a preference for the synthesis of rRNA. Of the *in vitro* transcript, 10% is rRNA whereas only 0.2–0.4% of the *E. coli* genome is complementary to rRNA. The ψ r factor is not required for, and does not stimulate, transcription of the rRNA genes. Hussy, Pero, Shorestein, and Losick have independently found that rRNA accounts for about 10% of the RNA transcribed from *B. subtilis* DNA by their most highly purified RNA polymerase holoenzyme from exponentially growing cells (personal communication).

The percentage of rRNA in the *in vitro* product observed in *E. coli* and *B. subtilis*, although relatively high, is below the possible *in vivo* value of 40%. It is difficult, however, to know what percentage to expect. *In vitro*, the polymerase may not recognize only the correct promoters, and the rRNA synthesis observed here may be artefactual. Even if the polymerase does read the correct promoters, this reading may not be at

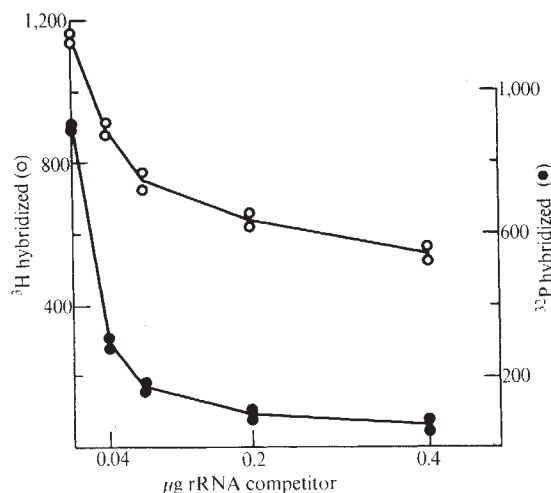


Fig. 6 Hybridization competition of *in vitro* labelled 3H -RNA by rRNA. The reaction was a scaled down (0.1 ml.) version of the reaction described in Fig. 3, containing 7 μ g of *E. coli* DNA, 2 μ g of RNA polymerase holoenzyme and 0.05 mM UTP 2,000 μ Ci/ μ mol. The reaction was stopped by adding 2 ml. of cold $2\times$ SSC and extracted once with 1 ml. of $2\times$ SSC saturated phenol. The aqueous phase was further diluted with 4 ml. of $2\times$ SSC until it contained 10^5 3H acid-precipitable c.p.m./ml. Then 1.2×10^4 c.p.m. of ^{32}P -rRNA 0.1 mCi/ μ mol in $2\times$ SSC were added to 1.2 ml. of the diluted aqueous phase. A sample of 0.1 ml. of the mixture was added to 0.7 μ g of single stranded *E. coli* DNA enriched for rRNA cistrons by elution from MAK column (Fig. 5) in 0.1 ml. of 0.05 M NaPO₄, pH 6.7, 0.7 M KCl. Increasing amounts of rRNA 40 μ g/ml. in $2\times$ SSC were added to the reactions and the final volume of all tubes adjusted to 0.210 μ l. with $2\times$ SSC. The hybridization reactions were incubated, RNA digested, filtered, dried, and counted as described in Fig. 3. With no competitor added, 70% of the input of ^{32}P -rRNA was retained by the filter and 12% of the 3H -RNA. All TCA precipitations and hybridizations were done in duplicate; 0.4 μ g of ribosomal RNA competitor reduced the amount of ^{32}P -rRNA bound to the filter by 95% and the amount of 3H -RNA bound by 61%. Backgrounds were 0.5% of the input ^{32}P -RNA and 1% of the 3H -RNA as determined by counts retained by the filters after hybridization in the absence of DNA. $\circ$, 3H ; $\bullet$, ^{32}P -rRNA.

the correct levels, since the *in vitro* system is stripped of all regulatory factors normally present in the cell. There are no known mutants in the promoters of the rRNA cistrons which can be used to test directly the correctness of the reading.

If initiation is occurring at the correct promoters, the 10% of rRNA in the total transcript may represent the full constitutive expression of the rRNA cistrons. This raises the possibility that rRNA cistrons are turned off by a repressor during the stringent response. These experiments do not, however, rule out the possibility that the rRNA genes are controlled in a positive manner, for the *in vitro* level of transcription may not reflect the full activity of the correct promoters. The assay methods described here are sensitive to small amounts of rRNA. Clearly they will be useful in a search for controlling factors for the synthesis of ribosomal RNA.

David Pettijohn has also found that up to 10% of the *in vitro* product RNA transcribed from *E. coli* DNA by RNA polymerase holoenzyme is rRNA (personal communication).

I thank W. Gilbert and K. Weber for advice and encouragement; J. Roberts, E. Minkley, R. Block and H. Ikeda for discussion, and S. S. Waisman and D. Bowen for technical assistance. R. Losick and J. Pero gave many helpful suggestions. I was supported by a predoctoral fellowship from the US National Science Foundation and by grants from the US National Institutes of Health.

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Received December 3, 1971; revised January 5, 1972.

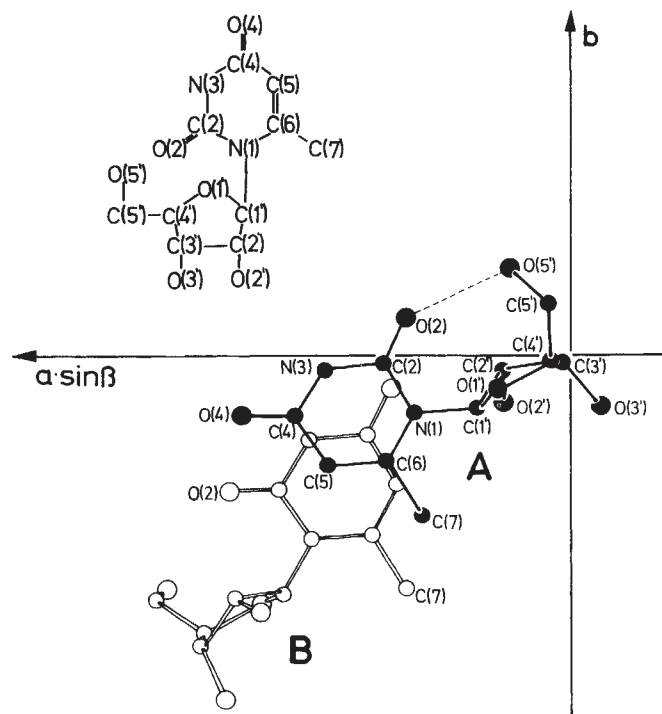


Fig. 1 Base stacking in 6-methyluridine viewed along the *c* axis.

in *anti* conformation. 4-Thiouridine may occur in *syn* conformation because of the stabilization by the peculiar hydrogen bonding and packing scheme or the particular C(3')-endo-C(4')-exo puckering* of the ribose unit. The correlation between conformation about the glycosidic bond and the sugar puckering in nucleosides was further studied by X-ray analysis of 6-methyluridine (Fig. 1). This species, according to nuclear magnetic resonance studies⁶, exists in the *syn* conformation in aqueous solution because of the bulky methyl group in position 6 of the pyrimidine ring.

6-Methyluridine was crystallized from aqueous solution to form colourless, thick plates of the monoclinic space group $P2_1$ with cell dimensions $a = 19.895_3$ Å, $b = 8.201_2$ Å, $c = 6.786_2$ Å, $\beta = 87.30_3^\circ$ and two molecules of the nucleoside within the asymmetric unit as derived from the measured density of the crystals (1.54 g/ml.). A total of 2,723 reflexions was measured on an automatic Stoe diffractometer with graphite-monochromatized $\text{MoK}\alpha$ -radiation ($\lambda = 0.70926$ Å); the structure factors were given weights proportional to $1/\sigma_{F_{\text{obs}}}$ derived from counting statistics plus a 1% of F^2 allowance for machine error, and data with $F_{\text{obs}} < 3 \cdot \sigma_{F_{\text{obs}}}$ were neglected. The structure was solved by means of cyclic application of the tangent¹⁰ formula combined with multiple solution techniques¹¹. The phase information of a starting set of four phases determined from Σ_1 relationships, four origin and enantiomorph determining and three further phases was extended to include all of the 247 reflexions with normalized structure factors greater than 1.6. With the phases derived from the most consistent¹¹ solution, we produced a Fourier synthesis (E-map) which clearly revealed the positions of the atoms of the two bases and their substituents O(2), O(4), C(7), C(1'). The atoms of the ribose unit were located from a subsequent Fourier synthesis and, after several cycles of least squares refinement, the positions of all hydrogen atoms except those on both of the O(5') hydroxyl groups could be determined from difference Fourier syntheses. The crystallographic agreement factor $R = \Sigma |F_{\text{obs}}| - |F_{\text{calc}}| / \Sigma |F_{\text{obs}}|$ for the 2,623 observed reflexions is 5.6%.

With a few exceptions, bond angles and distances within the two crystallographically independent molecules are very

* In ref. 9, the sugar conformation was called C(3')-endo but it is C(3')-endo-C(4')-exo instead, a puckering not previously observed.

Conformation of 6-Methyluridine — a Pyrimidine Nucleoside in the *syn* Conformation

IN a nucleoside the rotation of the nucleobase about the glycosidic bond relative to the sugar moiety is sterically hindered and two conformational ranges, *syn* and *anti*, are preferred¹. Theoretical²⁻⁵ and spectroscopic⁶⁻⁸ investigations suggest that the *anti* conformation is slightly more energetically favoured than the *syn*. In the crystalline state, this is also true for purine nucleosides but all pyrimidine nucleosides, except 4-thiouridine⁹ (a tRNA minor constituent), crystallize

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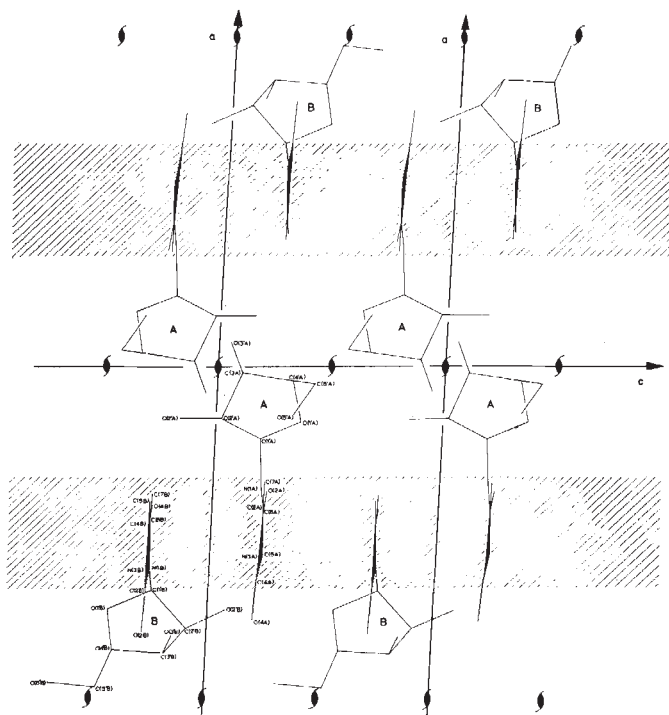


Fig. 2 Projection of the crystal structure down the *b* axis. Hydrophobic bands are indicated by hatching.

similar. The principal difference exists with respect to the location of atom O(5'). The O(5')-C(5')-C(4')-C(3') torsional angle is *trans* (179.8°) in molecule *B* but *gauche* (62.5°) in molecule *A*, rendering atom O(5') above this ribose unit. Both molecules exhibit the *syn* conformation with torsional angles C(2')-C(1')-N(1)-C(6) of 130.2° and 131.6° for molecules *A* and *B* respectively. Atoms O(2) and O(5') in molecule *A* are hydrogen bonded intramolecularly with an O(5')...O(2) interatomic distance of 2.827 Å; in molecule *B* such a hydrogen bond cannot form because of the *trans* conformation of the O(5')-C(5')-C(4')-C(3') torsional angle.

The intramolecular O(5')...O(2) hydrogen bond is not observed in either molecule, indicating that the *syn* conformation in 6-methyluridine might not be stabilized by a hydrogen bond, but by steric interactions due to the bulky methyl group.

The ribose moieties in both molecules are puckered C(2')-endo and this is apparent from both the endocyclic torsional angles and the deviations of the ribose ring atoms from the five different four-atoms least squares mean planes. Bond angles and distances are in reasonable agreement with data observed in other C(2')-endo puckered riboses¹⁰. As a result of the *syn* conformation of 6-methyluridine, the contacts between atoms O(2) and H(2') in molecules *A* and *B* are only 2.24 Å and 2.26 Å respectively. The corresponding distance in 4-thiouridine is 2.52 Å, but in this structure an additional unfavourably close O(2)...H(3') contact of 2.27 Å has been observed.

In 6-methyluridine, the exocyclic angles around the atoms of the glycosidic bond, N(1) and C(1'), are comparable with those in pyrimidine nucleosides in *anti* conformation⁹, while they seem to be strained in 4-thiouridine. Thus the 6-methyluridine molecule, although in the same *syn* conformation as 4-thiouridine, seems to be in a more relaxed form as is apparent from the normal C(2')-endo puckering of its ribose unit, while 4-thiouridine exhibits an unusual C(3')-endo-C(4')-exo ribose conformation. Dreiding wire model shows that the C(2')-endo sugar conformation should be favoured for a nucleoside in *syn* conformation as only in this case (and in the unlikely O(1')-endo form) is the nucleobase, and with it atom O(2), moved further apart from the sugar ring. The C(2')-endo and the C(2')-endo-C(3')-exo conformations

observed in *syn*-purine structures^{13,14} also suggest that the C(2')-endo or a related conformation is preferred for nucleosides in the *syn* conformation.

From the projection of the 6-methyluridine crystal structure down the *b* axis, the clear separation in alternating hydrophobic and hydrophilic zones extending parallel to the *b,c* plane can be seen. Within the hydrophobic bands around $a=1/4$ and $a=3/4$ the heterocyclic bases are arranged almost parallel to the *a,b* plane at $c=-0.22$ and $c=0.26$, forming stacks along the *c* axis with interplanar spacings of about 3.3 Å. (The overlapping of adjacent bases in the stack is shown in Fig. 1.) The ribose units form hydrophilic bands within the *b,c* plane around $a=0$ and $a=1/2$. It is remarkable that 4-thiouridine, which is also in the *syn* conformation, exhibits a packing scheme of the same general features, with alternating hydrophobic and hydrophilic bands built up from parallel stacked bases and the ribose units respectively.

We thank Dr Paulus for data, Professor F. Cramer for his interest and support, and Miss U. Wittenberg for technical assistance. We also thank Dr P. C. Manor for critically reading the manuscript.

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Received August 24, 1971.

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Relationship between the Stage of Development of Foetal Hearts and their Survival in Organ Culture

We required a method for the storage of animal and human foetal heart material which would enable us to study the possible use of foetal tissue in replacement surgery and to examine the interplay between implanted foetal cardiac tissue and the healing of experimental myocardial infarcts.

Wildenthal described a method in which functioning foetal mouse hearts could be studied *in vitro* for prolonged periods^{1,2}. We have repeated Wildenthal's experiments and obtained comparable results.

We tried other techniques for maintaining the hearts, including aortic perfusion with scaled-down heart/lung machines, and a rotating arm drawing the heart through the medium, aorta first, but all results were inferior to those

described by Wildenthal, whose technique was used throughout the work described here.

We set up series of experiments to relate the stage of development and the size of the foetal mouse hearts to survival time. We also measured the performance of the hearts by recording the heart rate at intervals during the culture period. The beating and survival of foetal hearts from man, dog, cat and rabbit were compared, and related to size and stage of development, using the same technique.

For the purpose of this investigation the stage of development is defined as a percentage of full term, and we have accepted the shortcomings of this relationship. If the complete hearts were too large for adequate diffusion through the specimen, they were cultured as slabs of tissue. The survival of slabs of heart tissue of different size obtained from the same foetal heart and the survival of slabs of tissue from hearts of different stages of development were also investigated, in an attempt to find the optimum size.

Nineteen-day foetal mouse hearts were cultured resting on stainless steel grids (mesh size 0.1 mm) just touching the surface of commercially prepared medium 199 (Burroughs Wellcome) + 35% colostrum-deprived calf serum (Burroughs Wellcome) + 50 µg/ml. insulin + 0.1 µg/ml. cortisol, in atmosphere of 95% oxygen and 5% carbon dioxide at a temperature of 37° C. This experiment differed from Wildenthal's only in that the single strength medium 199 instead of the dried 199 medium was used, and the Petri dishes were stacked on a frame in a 'Perspex' container.



Fig. 1 The culture chamber ready to place in an incubator at 37° C. The three small culture dishes containing 2 ml. of culture medium can be seen in each Petri dish. Each culture dish contains one heart on a stainless steel grid, large enough to support the heart. The O₂/CO₂ mixture is left to flush the chamber for 2 min before sealing.

To study the effect of the stage of development and the size of the foetal mouse hearts on survival time, hearts were cultured from 12, 13, 14, 15, 17, 18, 21-day foetal and also newborn mice. Results were expressed as a percentage of full term, which is 22 days in the strain of mice used. The number of foetal hearts in each group varied from nine to fifteen, and each group was composed of litter mates. The hearts which were still beating were cultured intact. It was hard to assess

the early stages of development because of difficulties in culturing the small, delicate 12, 13 and 14-day foetal mouse hearts, which tended to flatten and slip through the stainless steel grids, several wires penetrating through each individual heart. This was overcome by placing these tiny foetal hearts on small squares of lens paper³ on the grids. These hearts were not turned over every 48 h as is usual in this technique, to avoid damaging the delicate tissues.

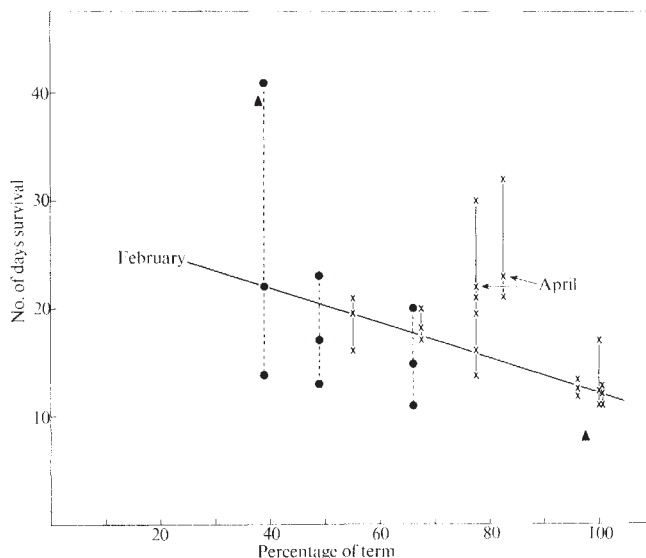


Fig. 2 The survival time of intact beating foetal hearts from mouse (x), rabbit (●), and cat (▲), related to the percentage of full term. The line goes through all the experiments except for two conducted in April, which show atypical results.

We used rabbit foetal hearts, each cut into different sized slabs to assess the relationship between survival and size of tissue. Beating was seldom observed in the tissue slabs, and death was defined using trypan blue, which enters and stains only dead cells. The vital stain neutral red was abandoned because it made the cells photosensitive. These stains tend to reduce survival and performance as both are slightly toxic. All slabs of tissue chosen for culture were from hearts that were demonstrably alive, having started to beat when dissected out and placed in the culture medium.

Our results were comparable with Wildenthal's, the 19-day foetal mouse hearts surviving and beating for 15.22 days.

Comparing the survival times of different intact foetal mouse, cat, rat and rabbit hearts of different ages, we found that younger and smaller foetal hearts survived for the longest times.

Two plots are shown in Fig. 2; the line through the average survival times of the foetal mouse hearts is labelled February and the individual results are labelled April. The difference is possibly due to the time of the year⁴⁻⁶, for in the spring the maternal ability to supply the foetus with reserves of enzymes, vitamins and other nutrients is greater. The difference may also reflect improvements in technique, due to repetition.

Slabs of tissue survived for shorter times than did intact hearts. Intact beating rabbit hearts (48% term) survived for 13–20 days, the average survival time being 16 days, compared with rabbit heart slabs (48% term from the same litter) which survived for 11–12 days, when they showed no sign of beating, or for 14–15 days, when beating was observed. Only three of the fifteen slabs were beating. We think this is probably related to the amount of conducting tissue present in the slab⁷⁻¹².

Fifteen slabs of non-beating foetal cat heart (41% term) survived for 13–23 days, an average of 17 days. We predict from our results that intact hearts would survive for approx-

imately 37 days. Hearts of 38% term survived for 39 days. Fourteen slabs of non-beating foetal dog heart (76% term) survived for 7–9 days, an average of 8 days. Thirty-nine slabs of non-beating foetal rabbit heart (66% term) survived for 9–16 days, the average being 12 days. The corresponding intact hearts averaged 15 days.

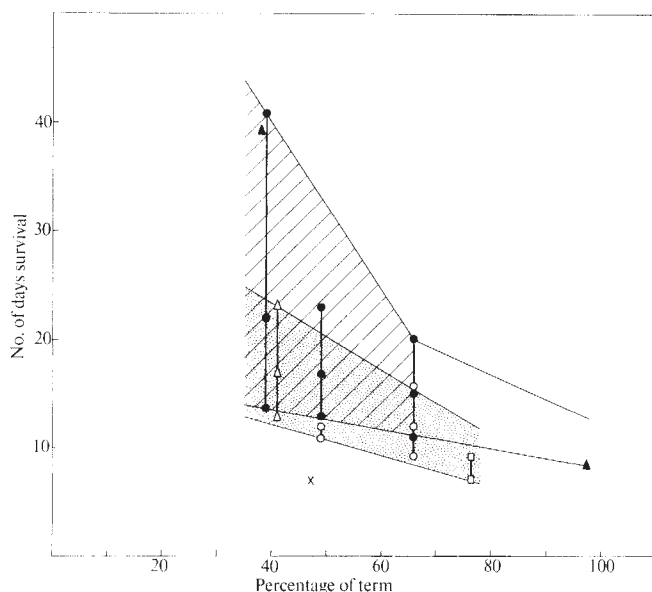


Fig. 3 The survival time of intact beating foetal hearts compared with the survival time for non-beating foetal heart slabs. (The intact foetal cat hearts all survived for 42 days.) The poor performance of the human foetal heart slabs is probably related to experiment circumstances. Intact foetal hearts: ●, rabbit; ▲, cat. Foetal heart slabs: ○, rabbit; △, cat; □, dog; ×, human.

Fourteen slabs of non-beating human foetal heart (47% term) survived for 7 days. We think this shorter period is related to the 3–6 h delay between obtaining the human tissue and setting up the culture, when storage conditions were not ideal.

Table 1 Survival of Heart Tissue related to Size

Animal	Area of tissue in contact with grid (mm ²)	Survival time (days)
Rabbit (66% term)	1–2	11–12
	3–4	11–12
	6.3	14
	9.6	14
Cat (41% term)	1–1.5	13
	3–3.6	13–23
	6.3	15–16
	8.8	19–23
	17.3	19
	20.3	19

To establish whether the survival time of slab was related to size or to the stage of development, specimens were cut into slices of the same thickness, but with different sized areas in contact with the tissue culture grids. The survival times (Table 1) revealed no remarkable relationship between size and survival.

We thank Miss Janet Way for technical assistance, the National Heart Hospital for facilities and equipment, Professor

M. G. H. Jukes for advice, and the British Heart Foundation and Boehringer Ingelheim Ltd for financial support.

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Received September 30, 1971.

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Calcium Release induced by Interaction of Angiotensin with its Receptors in Smooth Muscle Cell Microsomes

THE increase in calcium concentration which causes contraction of myofilaments may result from increased cellular permeability to extracellular calcium and/or translocation from cellular binding sites¹. We have now demonstrated that the interaction of angiotensin with specific receptors in smooth muscle cell microsomes increases the release of membrane-incorporated calcium, in support of the translocation theory. Microsomes were prepared from adventitia-free rabbit aortae in 0.32 M sucrose–0.01 M Tris maleate, pH 7.4, by differential centrifugations between 5,000 and 102,000g according to Verity and Bevan². The vesicular nature of the 102,000g fraction was established by electron microscopy. No cytochrome c oxidase activity³ was detected, thus demonstrating the absence of mitochondrial contamination. Calcium binding by these isolated vesicles was studied in incubation mixtures containing 100 mM KCl, 5 mM MgCl₂, and 10 mM histidine buffer (pH 6.8), with various concentrations of CaCl₂ (containing 0.6×10^{-3} μ Ci ml.⁻¹ ⁴⁵Ca), in 0.5 ml. Vesicular protein concentration⁴ was 50–100 μ g ml.⁻¹. The reaction, carried out at 26° C, was stopped by filtration through a 'Millipore' filter (HA, 0.45 μ m) followed by quick rinsing with 5 ml. of chilled calcium-free medium, and the amount of calcium retained by the membrane fragments measured by counting filters after dissolution in 'Cellosolve' in a liquid scintillation spectrometer. Calcium binding in vesicles reached equilibrium after 10 min. The addition of 5 mM ATP to the incubation medium increased the binding 4-fold. Direct measurements of calcium-stimulated ATPase showed that activation of this enzyme was associated with calcium binding (Table 1). Oxalate at a concentration of 2.5 mM increased the calcium uptake about ten-fold; this potentiating effect, also observed on microsomal vesicles of skeletal, cardiac and uterine muscle^{5–8}, is explained on the basis of its ability to permit trapping of calcium within the microsomes in the form of insoluble calcium oxalate. In the presence of ATP and absence of oxalate, calcium binding was saturated at a calcium concentration of 10^{-4} M in the medium. The calcium binding capacity was 6.5 pmol μ g⁻¹ protein. Sodium azide, known to inhibit calcium uptake in mitochondria⁹, had no effect on calcium binding by microsomes at a concentration of 0.5 mM. The

Table 1 Activation of Aortic Microsomal Adenosine Triphosphate by Ca^{2+}

	Minutes of incubation				
	1	2	3	5	10
Added $\text{Ca}=0$	0	0.1 ± 0.0	2.0 ± 0.3	1.8 ± 0.6	3.7 ± 1.2
Added $\text{Ca}=2 \text{ mM}$	0	1.1 ± 0.1	2.8 ± 0.6	5.3 ± 1.8	14.2 ± 3.8

Results are expressed in $\mu\text{mol P}_i \text{ mg}^{-1}$ vesicular protein. The assay contains 10 mM histidine (pH 6.8), 100 mM KCl, 5 mM MgCl_2 , 5 mM ATP, in a volume of 0.5 ml. Incubation temperature 26°C . Protein concentration $50\text{--}100 \mu\text{g ml}^{-1}$. Mean values $\pm$ s.d. are obtained from four experiments. Inorganic phosphate was determined by the molybdovanadate method¹⁴.

release of membrane-incorporated calcium was studied after loading vesicles with 10^{-4} M Ca for 10 min. The incubation medium was then diluted by the addition of 25 volumes of calcium-free medium and the amount of calcium remaining in the vesicles was determined at various intervals by the filtration technique. Half the incorporated calcium was released into the medium within 1 min. The addition of ATP to the medium increased the rate of release. Angiotensin II increasingly affected calcium release both in the presence and absence of ATP to a significant degree within the first 2 min (Fig. 1). After 5 min, the amount of bound calcium remained constant up to 30 min and identical in the absence and in the presence of angiotensin. This result suggests that in the experimental conditions used, only about 60% of bound calcium was rapidly released, and that angiotensin was only acting on this fraction. When the concentration of angiotensin II in the medium was greater than 10^{-7} M , the releasing effect of angiotensin reached a maximum corresponding to a 25% increase in the rate of release occurring in its absence (Fig. 2).

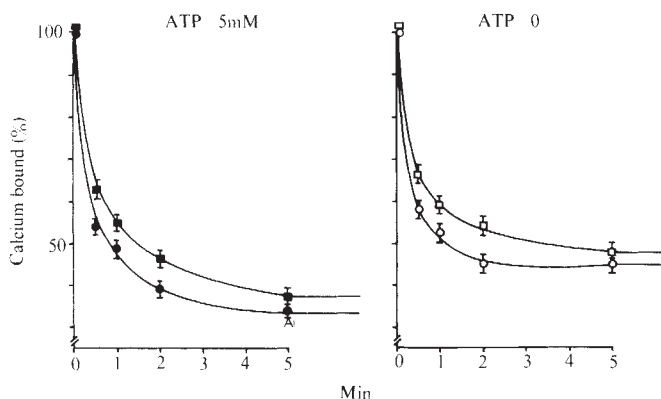


Fig. 1 Calcium release from microsomal vesicles previously loaded with 10^{-4} M Ca for 10 min. Each point represents the mean of from five to seven experiments. The squares and the circles refer to experiments performed in absence and in presence of angiotensin (10^{-7} M) respectively. Vertical bars indicate standard deviations. Control and angiotensin values are statistically different at times 0.5, 1 and 2 min (Student's t test— $P < 0.05$).

The binding of angiotensin to the microsomal vesicles was studied simultaneously with the use of ^3H -angiotensin II having a specific activity of $32.5 \text{ Ci mmol}^{-1}$ and retaining the full biological activity of the unlabelled hormone¹⁰. The incubation medium also contained 100 mM KCl, 5 mM MgCl_2 , 10 mM histidine buffer (pH 6.8) in a volume of 0.5 ml. Vesicular protein concentration was $50\text{--}100 \mu\text{g ml}^{-1}$. Incubation was performed at 26°C after which membrane-bound and unbound radioactivity were separated by means of dextran-coated charcoal¹¹, followed by rapid cooling at $+4^\circ \text{C}$. Membrane-bound tritium remained constant during a 3 min incubation with charcoal; the incubation time with charcoal used in this study was 2 min. The time-course of the radio-

activity incorporation into vesicles reached equilibrium after 30 s at an angiotensin concentration of $2.5 \times 10^{-10} \text{ M}$, favouring a reversible phenomenon. Cooling of the incubation medium to $+4^\circ \text{C}$ halved the amount of radioactivity incorporated. The concentration dependence of ^3H -angiotensin II binding to microsomal vesicles is shown in Fig. 2. Saturation of binding sites occurred in the range $1.0\text{--}3.2 \times 10^{-7} \text{ M}$. On the basis of the specific activity, the amount of angiotensin bound at saturation was calculated to be $35.4 \text{ pmol mg}^{-1}$ of membrane protein.

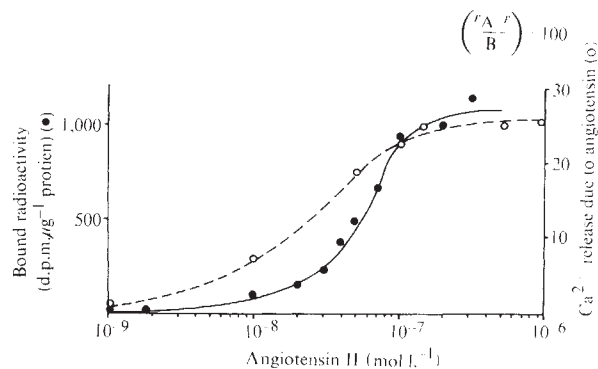


Fig. 2 Relationship between binding of labelled angiotensin and calcium-releasing effect to various concentrations of labelled and native angiotensin II. Incubation time with angiotensin was 30 s; r_A : calcium release after 30 s in presence of angiotensin; r : calcium release after 30 s in absence of angiotensin; B : bound calcium at beginning of release. Each point represents the mean of from five to seven experiments. These experiments were performed in presence of 5 mM ATP.

Three lines of evidence support the hypothesis that the binding sites present in the microsomal vesicles are those involved in the biological response of the hormone. First, the binding parameters found in the vesicles are very close to those previously found in our laboratory on whole rabbit aorta¹². The difference in the number of specific binding sites in aorta and in microsomes reflects the 1,000-fold purification occurring during the extraction of the vesicles. Second, a good relationship was found between the binding parameters and the biological responses induced by angiotensin both in aorta and in vesicles (Fig. 2; Table 2). Third, the addition of unlabelled angiotensin II to the medium reduced the incorporation of ^3H -angiotensin II, in proportion to the concentration of unlabelled ligand. No inhibitory effect on ^3H -angiotensin II binding was observed with 4 Phe-angiotensin II, 6 Ala-angiotensin II and 8 Ala-angiotensin II even at concentrations 1,000 times greater than that of the ^3H -angiotensin II. These substituted analogues failed to alter calcium release. This indicates that the aromatic amino-acids facing outside in the model of the angiotensin molecule recently proposed by two of us¹³ are implicated in the binding process triggering the biological action. L-Noradrenaline also failed to alter the binding of labelled angiotensin.

Table 2 Relationship between Binding Parameters and Biological Response of Angiotensin in Aorta and in Microsomes

	Binding parameters of ^3H -angiotensin II		Biological response	
	N of sites (moles)	Association constant	Maximum response	Half-maximum response
Aorta	$0.84 \times 10^{-14} \text{ mg}^{-1}$	$1.3 \times 10^{-8} \text{ M}$	10^{-7} M	$1.5 \times 10^{-8} \text{ M}$
Microsomes	$3.54 \times 10^{-11} \text{ mg}^{-1}$	$5.5 \times 10^{-8} \text{ M}$	10^{-7} M	$2.3 \times 10^{-8} \text{ M}$

Data on aorta were obtained in a previous study¹². Biological responses: aorta: contraction; microsomes: calcium release.

There is still some doubt about the origin of the microsomal fraction, which may derive from cell membrane, endoplasmic reticulum or micropinocytic vesicles². This investigation demonstrates, however, that specific angiotensin receptors are located in this fraction, and that a displacement of calcium from microsomal particles results from hormone receptor interaction. Like striated^{5,6} and cardiac⁷ muscle, the aorta possesses a membranous system capable of binding a large amount of calcium. The vasoactive angiotensin II releases calcium from this binding site and the resulting increase in cytosolic calcium concentration may account for the contraction of the myofilament.

This work was supported by the Centre National de la Recherche Scientifique. We thank Dr P. Fromageot and Professor F. Morel for their interest, Drs P. A. Khairallah and B. Riniker for the analogues of angiotensin, and Dr J. Bariety for electron microscopy.

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Embryonic Red Blood Cell Formation

RESEARCH in development and in cancer are two of the most hotly pursued areas of modern biology. Answers will come from many directions, but red blood cell formation (erythropoiesis) is an excellent and easily accessible model system for studies in development and cancer. In particular, erythropoiesis in embryos, *in vivo* and *in vitro*, allows us to look at the mechanism of determination and of differentiation of cells. Such studies also have an obvious relation to an understanding of proliferative and of other diseases of the erythropoietic system, particularly in man. The purpose of this note is to point out certain strikingly similar features of erythropoiesis in the embryos of man, mouse and chicken and in the metamorphosing larva of the bullfrog, features which might well be more widespread among animals.

For purposes of illustration, we can use erythropoiesis in the chick embryo. Here it is known (reviewed by Lemez¹ and by Wilt²) that erythroid precursor cells arise *in vivo* in the mesenchyme after about 24 h of incubation or even earlier, presumably by a process of induction by endodermal cells, as shown by a classical transfilter experiment by Miura and Wilt³. Early erythroid precursor cells appear in clusters and are morphologically identifiable in the light and electron microscopes⁴⁻⁹. Haemoglobin begins to be detectable after 36 h of incubation; a circulatory system forms at 48–60 h. The early embryonic haemoglobins seem to be the product of dividing and maturing cells of the so called "primitive" red cell series, a cohort of cells appearing as a group in the course of a day or so. Thus, the primitive cells tend to be at the same maturation stage at the same time. They have all stopped dividing by day 5; thereafter they continue to synthesize haemoglobin at a decreasing rate, circulate for a few days as mature red cells, carry out their oxygen transporting function in the embryonic circulation and then disappear^{1,2,4,10,11}; few are formed after day 13.

Their place in the circulation is taken after day 7 by the second and permanent red cell series^{1,2,4,10,11}—the morphologically

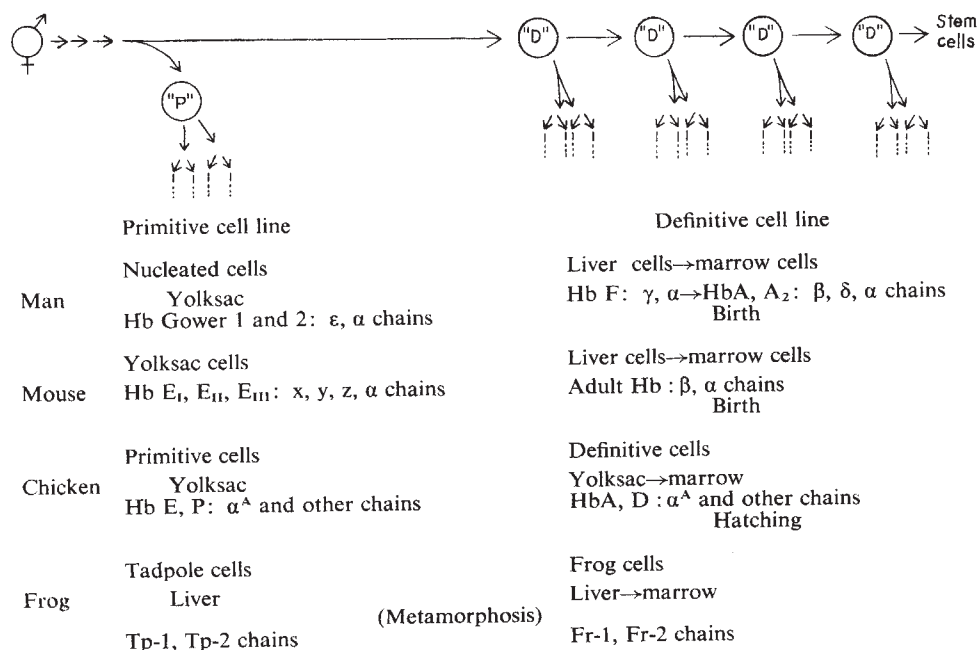


Fig. 1 A scheme for erythroid cell differentiation in the embryos and the adult of man^{14,15}, mouse⁸ and chicken (ref. 10 and unpublished results of J. L. Brown) and in the tadpole and the frog^{16,17}. The definitive cell line happens to change site of erythropoiesis from the liver to the marrow at about the same time as the change from HbF to HbA and A₂ production. Although correlated in time, the two events are probably not causally linked. Again, all three types of haemoglobin are produced by erythroid cells in either site, but their proportions change.

distinct definitive red cells carrying the adult haemoglobin types². In the chick embryo this is a self-renewing population, sequestered in the yolk sac tissue and later in the marrow, but separate from the circulation. The sequestered population contains the stem cells and the most immature stages of the definitive red cells. The cellular origin of the precursors of the definitive red cell line is quite unknown; their origin and the timing of the appearance of this second red cell line are prime targets of present day research. Organ culture experiments^{2,12} covering development to day 2.5, as well as organ culture¹³ and cell culture work in our own laboratory, covering also the appearance of the definitive red cell series and its haemoglobin, show promise that a biochemical approach to this system is possible.

It is instructive to compare embryonic erythropoiesis in the chick with that in man, mouse and frog, as shown in Fig. 1. The general conclusion can be drawn that in these four instances there are two distinct populations of erythrocytes, making their appearance early and late respectively during embryonic or larval development. In man, mouse and chicken, these populations are easy to distinguish on morphological grounds. Except in the frog, the early red cell is limited in duration, and seems to consist of a single cohort of cells, while the later cell line has a self-perpetuating stem cell line and in fact becomes the adult red cell population. There are also some interesting common features with regard to changes in the site of erythropoiesis, as noted in the figure. Furthermore, the "primitive" and "definitive" red cell lines in these four instances differ in the haemoglobins which they contain; in man, in the mouse and at least in the one well studied strain of chicken they have just one peptide chain in common between the haemoglobins of the two cell lines. In the tadpole-frog example, there are no common peptide chains. In man, further qualitative changes in haemoglobin pattern from haemoglobin F to haemoglobin A and A₂ take place in the definitive cell line. This further change is superimposed on and subsequent to the appearance of the definitive cell line. Less marked changes occur also in the definitive chick red cell line^{1,10}. No such secondary changes are known for the particular mouse or frog examples used in Fig. 1. Neither birth nor hatching seems to play a role in these changes.

The major point of interest in this scheme is the divergence between primitive and definitive progenitor cells ("P" and "D" cells in the figure); in other words the process of determination which is involved here. At least two possibilities exist in this model. The primitive and definitive cells might diverge as indicated from a common pluripotent precursor cell, the primitive cells as a single cohort, the definitive cells as a self-perpetuating cell line. Alternatively, it is possible that the immature primitive cells are themselves precursors of the definitive cell line. It is towards understanding this point, at a fundamental level, that much of the current research in this area is directed.

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Liposomes containing Antiviral Antibody can protect Cells from Virus Infection

CELLS in the body and in tissue culture can be protected from virus infection if they are bathed in fluids containing specific antiviral antibody. Except in rare instances¹, the antibody does not attach to the cells, so that protection is easily lost by dilution or washing². Antibody bound to bentonite particles can, however, protect cells from infection by coxsackie A-21 virus, and the protective effects are not lost by repeated washing². The particles seem to be held at the surface of the cells where they act as a protective shield.

By shaking some aqueous phase with a film of mixed lipids, Bangham³ produced lipid spherules, or liposomes, which consist of a closed system of concentric lipid bilayers alternating with aqueous compartments. The particles resemble natural membrane-bound structures^{4,5}. Liposomes constructed from normal red cell components can be lysed in a complement-mediated reaction that depends on the binding of anti-red cell antibody to red cell antigens incorporated into the particles^{6,7}. Proteins may be incorporated into liposomes⁸, increasing the spacing between lipid layers. Liposomes, which have been suggested as experimental models for lysosomes⁹, can interact with granules isolated from polymorphonuclear leucocytes resulting in the fusion of the two membrane systems and the release of lysosomal enzymes such as β -glucuronidase¹⁰. This interaction can be seen as a model for the fusion of phagocytic vesicles and lysosomes in the cell.

We have defined the conditions needed for the attachment of liposomes to cells in culture and demonstrated protection of the cells from virus infection by liposomes containing antiviral antibody.

The preparation of human IgG with a high neutralizing titre to coxsackie A-21 virus and iodination to give ¹²⁵I-IgG has been described before².

The molar ratios of lipids used were those given by Kinsky *et al.*¹¹ and Haxby *et al.*⁶. Small batches were made by dissolving 2.7 mg of sphingomyelin (3 μ M), 0.58 mg of cholesterol (1.5 μ M), and 0.33 mg of dicetyl phosphate (0.66 μ M) in chloroform with a small amount of methanol. The solution (usually in 4–6 ml.) was added to a 50 ml. round bottomed

Table 1 Incorporation of Cholesterol and IgG into Liposomes

Aqueous phase	% of added component in liposomes		% attached to cells
	³ H-Cholesterol *	¹²⁵ I-IgG	
0.3 ml. saline	7.8	—	< 0.5
0.3 ml. 5% IgG	22.0	N.D.	< 0.5
0.3 ml. ¹²⁵ I-IgG	N.D.	13	< 1.0

Each lipid film contained 3 μ M sphingomyelin (from bovine brain, Pierce Chemical), 1.5 μ M cholesterol (Sigma) [$\pm$ total c.p.m. = 4.9×10^6] and 0.66 μ M dicetyl phosphate (K and K Laboratories). The ¹²⁵I-IgG (14 O.D. units of protein) contained 9.4×10^3 /c.p.m.

* Liposomes containing ³H-cholesterol were dialysed overnight before washing by centrifugation.

Table 2 Preparation and Antiviral Activity of Antibody-containing Liposomes prepared with Various Amounts of Stearylamine in the Lipid Phase

Stearylamine μM	% ^{125}I -IgG incorporated into liposomes	Calculated antibody titre incorporated *	% adsorbed to cells	% expected antibody titre retained *	% plaque reduction
0	9.4	100	33	9.7	23
0.1	15.3	240	35	17.8	74
0.2	17.6	300	63	18.5	Toxic
0.1 †	16.2	280	38	18.2	73

Amounts of sphingomyelin and cholesterol were the same as given in Table 1. The final preparations were taken up in 0.3 to 0.4 ml. saline, and 0.05 ml. was used for the cell adsorption test. Similar values were obtained using 0.1 ml.; 0.1 ml. was used in the plaque reduction assay.

* Serial two-fold dilutions of liposomes or IgG (0.7 ml.) were incubated overnight at 6° with gentle shaking with 200 p.f.u. coxsackie A-21 virus (0.7 ml.). Duplicate 0.5 ml. aliquots were plated for plaque assay. The antibody titre was expressed as the reciprocal of the dilution resulting in a 50% reduction in p.f.u.

† Also contained 0.2 μM dicetyl phosphate.

flask and the solvent evaporated on a rotary vacuum evaporator at room temperature. The flask was flushed with N_2 and 0.3 ml. of the aqueous phase (saline plus the indicated additions) added. The lipid film was hydrated and then dislodged from the glass by the use of a Vortex junior mixer (Scientific Industries, Inc., Queens Village, New York). When suspended, liposomes were diluted with 4 ml. of saline and pelleted by centrifugation in a No. 50 head in a Spinco ultracentrifuge at 30,000 r.p.m. for 20 min. The pellet was resuspended and centrifuged twice more and taken up in 0.3 ml. of saline.

Liposomes (0.1 ml.) plus 0.3 ml. of Hanks saline (HS) were added to washed monolayers of ML cells growing in 60 mm Petri dishes². After 1.5 h at 37° C, unadsorbed liposomes were removed by two washes with HS. Cell pellets were scraped from the dishes, dissolved and the radioactivity determined. Inhibition of virus replication was determined on similarly treated monolayers by challenge of the washed cells with 50–100 p.f.u. of coxsackie A-21 virus. The monolayers were incubated for 1 h with the virus, overlaid with agar-containing medium and returned to 37° C. The plaques were read after 2 days².

Table 1 shows that human IgG in the saline phase improved the retention of cholesterol by the particles. An approximate two-fold increase in the volume of the final liposomes was seen. However, the particles did not become attached to cells in tissue culture. The composition of the lipid film was altered in an attempt to improve uptake by the cells. Dicetyl phosphate was omitted and replaced by stearylamine. Protein was incorporated into the liposomes as before (14.2% incorporated) and the particles attached to the cells of the monolayers almost instantly as they were added to the cultures, presumably as a result of electrostatic attraction between the positively charged particles and the negatively charged cells. Much (77%) of the added radioactivity in the liposomes became cell-associated.

Table 2 shows the results obtained with liposome preparations prepared with various amounts of stearylamine in the lipid phase. The amount of protein incorporated decreased when the amount of polar lipid was less than 0.1 μM (see also ref. 9). The percentage adsorbed to cells increased with increasing stearylamine, but 0.2 μM stearylamine resulted in particles which were toxic to the cells. From the amount of ^{125}I -IgG incorporated and the known anti-coxsackie A-21 titre of the original ^{125}I -IgG, the amount of specific antibody in the liposomes could be estimated (column 3, Table 2). Liposomes were tested for antiviral properties in two ways. Serial dilutions of the particles were incubated with virus to determine the direct neutralizing capacity of the complexed antibody (column 5, Table 2). The more active preparation⁵ showed that 18% of the antibody was available for virus neutralization. This figure resembles the amount of intrinsic enzyme activity found for intact liposomes containing lysozyme⁹, and may indicate that some of the protein in these particles is arranged at or near the surface inter-

digitated with the lipid film. The second test for antiviral activity measured the protection afforded to cell monolayers by attached liposomes. Reduction of the number of virus plaques was measured. A sample (0.1 ml.) of the liposomes made with 0.1 μM of stearylamine reduced the number of plaques by 74% (Table 2). On the basis of antibody titre incorporated into the particles, the liposomes were five to ten times as effective in the plaque reduction assay as were IgG-bentonite particles studied earlier². Similarly, the liposomes were 3,000–10,000 times as active as free IgG in a similar test². The free antibody was ineffective because it was almost all removed by the buffer rinses given before virus challenge. We conclude that virus infection can be suppressed effectively by antibody-containing liposomes held at the cell surface.

Various therapeutic applications are possible for drug-containing particles. The liposomes should be rapidly taken up by the cells of the reticuloendothelial system, including the phagocytic cells of the liver, and might prove to be an effective means of specific drug delivery for treatment of diseases in these areas. A great deal of attention is now being directed to such problems as gene therapy¹² and enzyme replacement¹³ for individuals suffering from inborn errors of metabolism. Two important considerations in these approaches are to prevent metabolic breakdown of the gene or enzyme to be incorporated and to deliver these materials to the desired area. It may be possible to modify the size and surface properties of liposomes so that they will be taken up specifically by the desired target organ, while at the same time retaining full advantage of the protective phospholipid coating.

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K⁺ Uptake of Guard Cells stimulated by Fusicoccin

CONSIDERABLE evidence has accumulated recently indicating that potassium ions are required for stomatal opening in the light¹⁻³. The observation that potassium ion accumulation occurs against a concentration gradient implicates an ion "pump"; ATP derived from photophosphorylation has been suggested as the energy supply. For some years, the metabolic and hormonal regulation of stomatal apertures has been known and used to understand mechanisms of stomatal behaviour⁴. However, the effect of these hormones and metabolic inhibitors on the potassium balance of guard cells is not known. In some species stomatal opening also occurs in the dark. Much less is known about dark opening than light opening and accumulation of potassium in guard cells during opening in darkness has not been demonstrated.

Fusicoccin is the primary phytotoxin produced by *Fusicoccum amygdali* Del., the pathogen of almond and peach canker⁵. The toxin has been isolated, characterized and shown to produce symptoms in the host similar to infection by the fungus^{5,6}. Dilute concentrations of the toxin introduced into the xylem or sprayed on to leaves increased the rate of water

loss⁵ and stomatal opening in a range of species both in the light and in the dark^{7,8}.

To test if the stimulation of stomatal opening by fusicoccin was accompanied by the accumulation of potassium ions in the guard cells, I treated epidermal strips and attached epidermes with fusicoccin in both the light and dark, and determined the accumulation of potassium ions in the guard cells by two methods. Also, fusicoccin was used in conjunction with metabolic inhibitors to suggest possible sources of energy for potassium ion uptake.

First the rate of opening of the stomata in light and darkness was established. Epidermal strips were removed from the abaxial surface of the primary leaves of bean (*Phaseolus vulgaris* L. variety Pinto) plants equilibrated in a room maintained at 27° C and 55% relative humidity and either illuminated at 50 W/m² (395-695 nm wavelength) for 3 h or kept in the dark for 11 h. The strips were immediately floated on a solution of (a) 10 µM fusicoccin in 0.02% (v/v) aqueous methanol and 10 mM potassium chloride (fusicoccin), or (b) 0.02% (v/v) methanol and 10 mM potassium chloride (control); the strips were maintained in the same light as the plants from which they were peeled. Three strips were removed from each solution after 0, 1, 1.5, 3, 4.5 and 6 h and the apertures of ten randomly-selected stomata per strip were quickly measured by light microscopy. The stomata of epidermes floated in fusicoccin opened wider than the controls both in the dark and in the light. In the light, the stomata opened from a mean aperture of 2.8 ± 0.2 µm when stripped to a maximum of 8.6 ± 0.3 µm 1 h after placement in fusicoccin. In the dark, the mean aperture of the stomata when stripped was 1.4 ± 0.2 µm. The opening was slower, however, reaching a maximum of 9.2 ± 0.4 µm after 3 h in the fusicoccin. The stomata floated in the control solutions for 1 h in the light and 3 h in

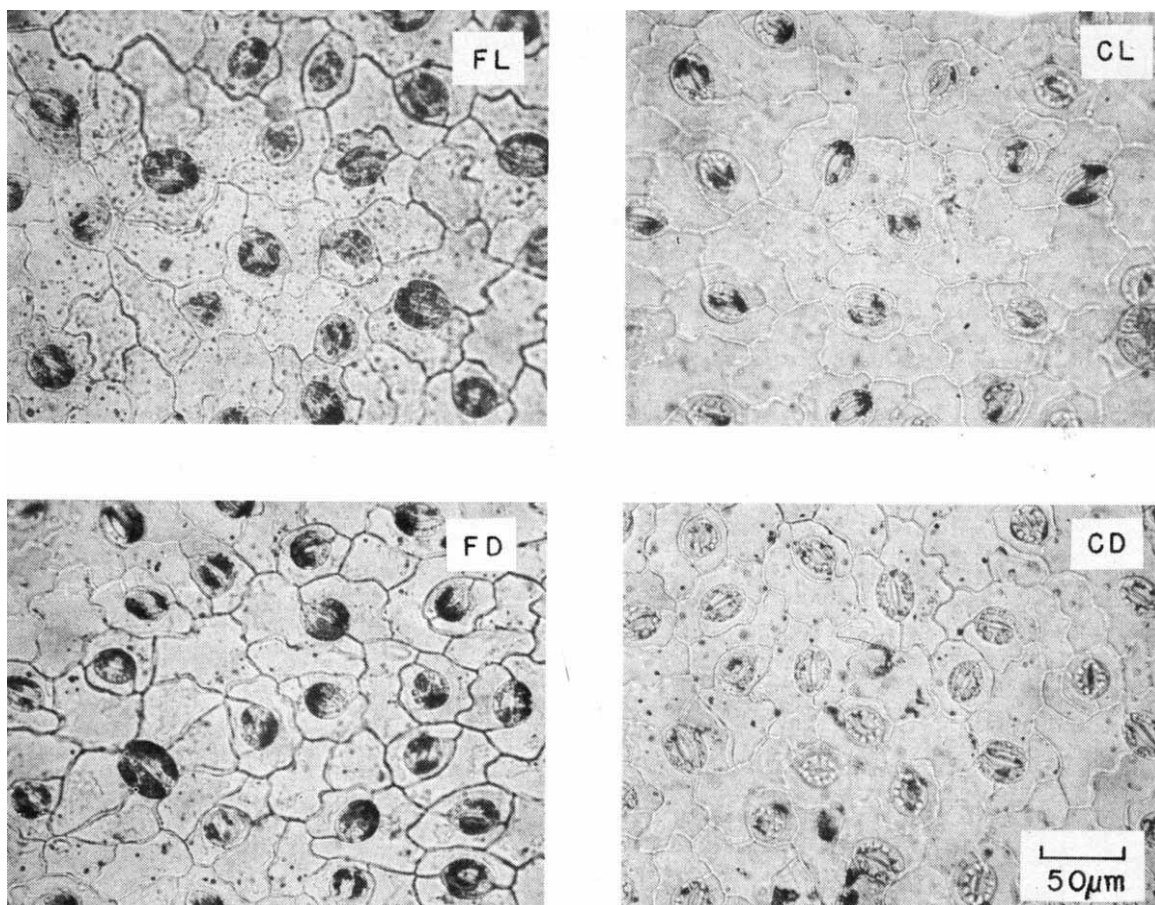


Fig. 1 Epidermal strips stained with a modified Macallum's method⁹ to identify the location and amount of potassium. L were floated for 1 h in the light, D were floated for 3 h in the dark in either 10 µM fusicoccin in 0.02% (v/v) aqueous methanol and 10 mM potassium chloride (F), or 0.02% (v/v) methanol and 10 mM potassium chloride (C). Staining caused the stomata to close; before staining the mean apertures were FL, 8.4 µm; CL, 5.2 µm; FD, 9.1 µm, and CD, 1.6 µm.

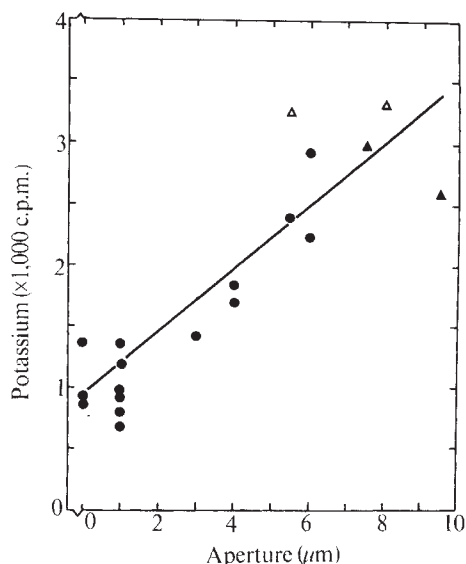


Fig. 2 Relation between concentration of potassium (c.p.m.) in guard cells and stomatal aperture for leaves painted with (a) 10 μ M fusicoccin in 0.02% (v/v) aqueous methanol and 0.05% (v/v) 'Triton X-100' in either the light (Δ) or dark ($\blacktriangle$), or (b) 0.02% (v/v) methanol and 0.05% (v/v) 'Triton X-100' in both the light and dark ($\bullet$). The concentration of potassium was measured in counts/10 s with an electron microprobe³ focused to 2 to 5 μ m on a reference crystal of scheelite and adjusted to give a voltage of 20 kV, a current of 9 mA and a specimen current of 50 nA.

the dark had apertures of 4.6 ± 0.2 μ m and 1.8 ± 0.3 μ m, respectively. The mean rates of stomatal opening from three sets of similar observations indicated that the fusicoccin-stimulated opening was approximately twice as fast in the light as in the dark.

As soon as the maximum opening had been achieved (after 1 h in the light and 3 h in the dark), a second series of strips were removed from the solutions and the potassium in the epidermes was stained with a modified Macallum's method in which the potassium is precipitated as black grains⁹. The strips floated on fusicoccin had high concentrations of potassium in the guard cells both in the dark and in the light, whereas the strips floated on the control solution had no detectable potassium in the guard cells in the dark and an intermediate concentration in the light (Fig. 1). Clearly the concentration of potassium in the epidermal strips floated on fusicoccin was greater than in the controls in both dark and light.

To establish that this was not simply a phenomenon limited to epidermal strips floated on fusicoccin, the concentrations of potassium in attached epidermes treated with fusicoccin were measured with the electron microprobe³. The primary leaves of bean plants, maintained as in the previous experiment, were painted with either 10 μ M fusicoccin in 0.02% (v/v) aqueous methanol (fusicoccin), or 0.02% methanol (control). 'Triton X-100', 0.5% (v/v), was added to wet the leaves. At 0, 3 and 7 h after painting, epidermal strips were quickly peeled from the abaxial leaf surface, immediately placed on a glass slide, and frozen by immersion in a mixture of dry ice and isopentane for 30 s. The frozen tissue was then freeze-dried under vacuum for 30 min. The potassium concentration as counts/10 s, at four positions in a pair of randomly-preselected guard cells and four points in the surrounding subsidiary cells, was measured with the electron beam of the microprobe focused to a diameter of 2 to 5 μ m³.

The relation between the potassium concentrations of the guard cells and the stomatal aperture was first established for the controls in both the light and dark. As previously observed in tobacco³, the concentration of potassium was linearly related to stomatal aperture over a wide range of apertures; the regression between potassium counts (P , c.p.m.) and aperture (A , μ m) was $P = 258 A + 900$ ($r^2 = 0.80$) as shown in Fig. 2. Paint-

ing the leaves with fusicoccin increased both the stomatal aperture and the potassium concentration in the guard cells; the addition of data from guard cells painted with fusicoccin in the light or in the dark 3 or 7 h earlier did not significantly alter the regression relating potassium to stomatal aperture ($P = 258 A + 930$). The concentration of potassium in the subsidiary cells was not affected by an increase in stomatal aperture in the controls or fusicoccin treated leaves. Clearly, the opening of the stomata by the fusicoccin is accompanied by an influx of potassium into the guard cells in attached epidermes as well as in epidermal strips. Moreover, potassium accumulation is not restricted to stomatal opening in the light, but can also operate in the dark. The results strongly suggest that biochemical and hormonal control of stomatal opening may be mediated by K^+ fluxes.

Finally the source of energy for the observed accumulation of potassium was investigated. To test whether oxidative phosphorylation was involved, 0.1 mM of 2,4-dinitrophenol was added to the solutions in experiments otherwise conducted as described previously. At a pH of 5.7, the 2,4-dinitrophenol inhibited the opening of the stomata in epidermal strips in both the light and dark, suggesting that oxidative phosphorylation may act as one energy source. The more rapid opening of the stomata in the light suggests that photophosphorylation provides an additional energy source. An inhibitor of open-chain photophosphorylation in chloroplasts, 3-(4-dichlorophenyl)-1,1-dimethylurea, has been used to ascertain the effect of open-chain photophosphorylation in stomatal opening^{10,11}. The addition to the solutions of 10 μ M of 3-(4-chlorophenyl)-1,1-dimethylurea, a closely related compound acting in a similar manner, reduced the rate of stomatal opening in the light of both the controls and fusicoccin-treated epidermes, but the inhibitor did not change the magnitude of the differences in aperture between the controls and treated stomata. This suggests that the additional source of energy may be provided by cyclic photophosphorylation.

It has therefore been clearly demonstrated that the phyto-toxin, fusicoccin, stimulates the uptake of potassium by guard cells. Moreover, similar concentrations of potassium were observed in guard cells opened to the same extent in the light and in the dark by fusicoccin. This strongly suggests that potassium plays an essential role in stomatal opening, even in unusual circumstances, and lends support to the potassium ion "pump" hypothesis of stomatal movements^{2,3}. Furthermore, the use of metabolic inhibitors and the observed differences in rates of opening and potassium accumulation in the dark and light suggest that a single energy supply cannot account for the uptake of potassium. So far, however, we can only speculate on how the fusicoccin increases potassium uptake. Although it is structurally different, conceivably the toxin may act in a manner analogous to certain cyclic antibiotics and polyethers that induce the active transport of potassium across membranes in animal mitochondria¹².

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Received August 23; revised September 20, 1971.

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BOOK REVIEWS

Foraminiferology

Manual of Planktonic Foraminifera. By J. A. Postuma. Pp. 420. (Elsevier: Amsterdam, London and New York, 1971.) \$26.50.

THE planktonic foraminifera are morphologically unspectacular. Their skeletal structure is relatively simple and their external shapes differ but little between species. During the past two decades, however, industrial micropalaeontologists (initially forced to make what use they could of the fossil assemblages present in their exploration areas) have proved by intensive study that these microfossils can provide a practical and detailed biostratigraphy of Cainozoic and late Mesozoic marine sediments. Now, nobody concerned with the stratigraphy of such rocks, especially in tropical and subtropical areas, can escape involvement with them. They also provide the best documented evolutionary lineages of any group of fossils. Publications multiply rapidly, and so do nominal species, suggested lineages and zonal schemes. These, together with the care and practice needed to recognize the often subtle specific distinctions, have created a real and obvious need for a working manual for species recognition coupled with a biostratigraphic key. Most large petroleum companies have produced their own manuals or indexes for internal use. That compiled for Shell has formed the basis of this volume.

Some 160 species of the Globigerinacea (the Heterohelidae are omitted) are described and illustrated here. Two facing pages are devoted to each species. On one, large optical photomicrographs of dorsal, ventral and axial views (often of topotypes) are juxtaposed against half-tone drawings of the same specimen in the same aspects, and are accompanied by a retouched photograph of an axial thin-section. The opposing page has brief notes of the type bibliographic reference, type locality, morphology and supposed stratigraphic distribution of the species.

The species are arranged alphabetically in three separate sections, one each for the Albian–Maastrichtian, Palaeocene–Eocene and Oligocene–Recent intervals. Each section contains both a fold-out range-chart and some photomicrographs of characteristic assemblages in thin-sections of pelagic micrites. Forty-eight zones have been adopted, modified or elaborated from previous publications and are formally defined (without type localities) for the Albian–Recent interval. The plan of the work is excellent (although scanning electron micrographs would have been preferable to drawings) and the publication standard (paper quality, typography, reproduction, and so on) is very high.

Unfortunately, the specialist will find something with which to quarrel on virtually every page. The whole work is an over-simplification. There are surprising omissions (even *Globigerina bulloides*!), many of which could lead to misidentification even of listed species (for example, *Globorotalia inflata* of the Neogene cannot be compared here with its Palaeogene analogue, *G. centralis*). Separation of Mesozoic and Cainozoic genera into different identification keys neatly avoids the difficulty of distinguishing different but similar forms of different ages (no clue is provided to distinguish, say, *Praeglobotruncana* from *Globorotalia*, or *Globigerinelloides* from *Hastigerina*). Some generic diagnoses are simply and objectively wrong—for example, *Hastigerinella* is not trochospiral, and the species placed here ("*H. bermudezi*") is a *Clavatorella*. Many stratigraphically useful genera, often of wide distribution, are omitted. To assign *Pseudohastigerina micra* to *Hastigerina* is retrograde, taxonomically and biostratigraphically. None of Pessagno's carefully defined Cretaceous taxa¹ is mentioned. At specific level, the list of omissions and corrections could be very long. Virtually no references are given to even the most important systematic or stratigraphic revisions even of the species illustrated. When synonyms are cited at all, they

reflect the state of knowledge of ten years ago. Some notes are patently absurd—for example, the author doubts that *Cassigerinella chipolensis* "belongs to the group of planktonic foraminifera in view of the imperforate wall structure", when the illustrations clearly (and correctly) show the wall to be perforate and, in any case, perforations and habitat are only indirectly related; the doubtful affinity of this genus to the Globigerinacea, because of its internal toothplate, appears from neither text nor figures.

The formal descriptions are largely superfluous. They are repetitious (for each species we are told that the wall is perforate, and so on) yet superficial; they tell nothing that cannot be seen in the pictures. No differential diagnoses of species are given, and comparative remarks are few. The "remarks" are frequently confined to a note of the provenance of the figured specimen (which, being often in the form "DB 272, Trinidad" or "Tschopp 3181, Cuba", may be useless except to those readers with access to Shell files). Not even the type stratigraphic horizons are cited, even though these could have been transcribed from Ellis and Messina's *Catalogue*² as easily as the type reference and the type geographic locality. And, yet, half of each text page of the systematic sections remains blank.

This could have been a most valuable book. With more compilative care and (perhaps) more expert knowledge, it could have been a standard work of reference. As it is, both industrial and academic micropalaeontologists must still rely on their own resources—if they are fortunate, to the first two volumes of the *Catalogue*² or to organization files; if not, to the welter of publications which still remains adequately to be assessed and summarized.

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² Ellis, B. F., Messina, A. R., Charmatz, R., and Ronay, L., *Catalogue of Index Smaller Foraminifera*, 1 (1968), 2 (1969). (Spec. Publ. Amer. Mus. Nat. Hist., New York.)

Mediaeval Archaeology

Scientific Methods in Medieval Archaeology. Edited by Rainer Berger. Pp. xvii+459. (University of California: Berkeley, Los Angeles, and London, July 1971.) £9.50.

THE aim of this compendium of papers is to illustrate how scientific techniques might be used in research on mediaeval Europe and the Near East. The majority were presented at an international conference in 1967 which was attended by scientists, archaeologists and historians. The book is divided into two parts, devoted to dating and "tracing" methods respectively.

In part one, ten chapters are devoted to techniques relevant to the study of timbers from surviving mediaeval buildings in Europe. Radiocarbon dating is described and the possible reasons for the discrepancies between radiocarbon ages and true calendar ages, as determined by dendrochronology, are outlined. The radiocarbon dating results for a group of mediaeval buildings are discussed in detail, a lavish sequence of photographs and architectural drawings of the buildings being included. The principles of dendrochronology are presented and in addition the typological development of timber-framed buildings and timber roofs in England is described. The remaining four chapters deal with thermoluminescent dating of ceramics, archaeomagnetic dating of pottery kilns and tephrochronology (that is, the study of the layers of airborne ash produced by volcanic eruptions).

The six chapters in the second part are concerned principally with the description of various physical methods, such as neutron activation and X-ray fluorescence analysis, for the examination of archaeological artefacts. Discussion of the use of these methods for identifying the raw materials used in the manufacture of glass, glaze and metal artefacts, for establishing the source of the clay used for pottery and for elucidating the techniques used in the manufacture of Islamic glass is included. There are also chapters on the possible determination of the intensity of worldwide lead smelting, using lead aerosol deposits in polar snow strata, and on magnetic prospecting for the location of buried archaeological features.

The main criticism of this book is that the choice of topics results in a somewhat unbalanced picture of both mediaeval archaeology itself and of the application of science to archaeology. First, undue emphasis (approximately two-thirds of the book) is placed on the study of surviving timber buildings and the scientific methods for dating them. Second, there is no mention of those techniques (for example, petrological and metallographic microscopy) which

are appropriate for the study of the development of ceramic and metal technology. Third, no adequate discussion of the role of the biological sciences in providing information on the environment (for example, flora and fauna) and on man himself (for example, ages at death, prevalent diseases) is included.

Thus although the conference itself was undoubtedly valuable in that it brought together both scientists and archaeologists and served to emphasize the importance of scientific and archaeological techniques for supplementing the data obtained from written records from the mediaeval period, the conference proceedings, published in this book, are of considerably less value. The mediaeval archaeologist would therefore be better advised to purchase one of the more general books, now available, on the application of science to archaeology, especially considering the high price of this volume.

M. S. TITE

Sperm Phylogeny

Comparative Spermatology. Edited by Baccio Baccetti. (Proceedings of an International Symposium held in Rome and Siena, 1/5 July 1969.) Pp. 573. (Accademia Nazionale dei Lincei: Rome; Academic Press: New York and London, 1970.) \$22.50; £10.50.

THIS volume records the proceedings of an international symposium which was designed to bring together morphologists, histochemists and physiologists to attempt to correlate structure and function of spermatozoa and to examine the available information from a phylogenetic standpoint.

The book comprises, therefore, individual contributions from specialists from widely differing disciplines and, as one whose interests lie particularly with the interrelationships of sperm structure and function, I found it of great interest to look at the subject through other eyes. I am left with little doubt that the symposium met a genuine need and that the book is a worthy record of its proceedings.

For taxonomists there is a wealth of information about the ultrastructure of spermatozoa of a wide range of invertebrate and vertebrate species with an important contribution by Irene Manton on plant spermatozoids. As an outsider, I was interested in her comments about the dangers of trying to extract from the evidence conclusions about phylogenetic relationships and of being trapped into absurdities "such as deriving lungfishes from mosses".

In addition to morphological and ultrastructural patterns a number of contributions are concerned with detailed studies of separate components

and organelles. Among those receiving individual treatment are the acrosome, the nucleus, the flagellum, the connecting piece, the post-nuclear cap, microtubules and mitochondria.

Functional aspects of ultrastructure which are discussed include both motility and fertilizing capacity. There are several contributions which illustrate the very successful application of histochemical methods at the ultrastructural level.

The quality of the illustrations, which form such a large part of this book, will undoubtedly make for its success; and the transcript of the discussions which followed each paper is a valuable addition to the text.

With the reservation that the book caters for very specialized interests, I support the claim made for it that it will be of value to research workers in zoology, reproductive developmental biology, anatomy, biochemistry, medical research and botany. J. L. HANCOCK

Microbes Against Pests

Microbial Control of Insects and Mites. Edited by H. D. Burges and N. W. Hussey. Pp. xxii+861. (Academic Press: London and New York, March 1971.) £11.50.

THE science of insect pathology has been a remarkably late developer. Born in the 1870s with the work of Pasteur and Metchnikoff, it made (apart from the excellent work of Dutky and his associates on *Bacillus popilliae*) little real progress for the next seventy years. The 1950s saw both a revival of interest and a necessary change of emphasis from empirical field trials of microorganisms for the control of insect pests to fundamental studies of the pathogens and their host-relationships. In 1963 Academic Press published a two-volume treatise entitled *Insect Pathology*, under the editorship of the late E. A. Steinhaus, which bore witness to the rapid progress engendered by the new approach. The same publishers have now brought out another major work in this field covering the developments of the past eight years.

The new book differs from its predecessor in being primarily concerned with the utilization of microorganisms for the purposes of pest control, but the importance of the biological background is fully recognized and much of the most interesting material is concerned with fundamental studies, particularly in the field of bacterial toxicology. The forty-three contributors who have collaborated in this work are acknowledged leaders in their fields and the contributions are, therefore, authoritative.

The uses of bacteria, viruses, fungi,

protozoa, rickettsiae and nematodes as agents of control are reviewed in turn and separate chapters are devoted to the control of arthropod groups of particular economic importance. Four chapters are allotted to *Bacillus thuringiensis*, the most widely used pathogen, which allows for an adequate treatment of modern developments in the study of its complex array of toxins. The remaining chapters deal mainly with more general topics, including the identification of pathogens, synergism by chemical insecticides, host resistance, the influence of environmental factors on control and the safety of insect pathogens with respect to man, other vertebrates and beneficial insects. The problems of commercial exploitation are covered in chapters on large-scale propagation, formulation, standardization and assay, strain improvement and economics. A final chapter evaluates the advantages and disadvantages of microbial methods and forecasts their likely role in future pest control practice.

Most of the articles in this book are interestingly, and often stimulatingly, written and the general student of biological control will find much of value. Nevertheless, it is to the specialist worker in insect pathology that the book is primarily addressed and no less than eighty pages have been devoted to appendices tabulating information of use to him. Some are bibliographic, listing literature sources—a valuable feature in a subject whose literature is widely dispersed. Others name the laboratories from which pathogens, antisera and standard strains of test insects can be obtained.

Much of the value of a book of this type depends on the completeness of its bibliographies and most chapters conclude with very exhaustive lists of references. Although, as one contributor points out, the relevance of a paper is not necessarily apparent from its title, it is equally true that the practice of listing references without titles greatly reduces the value of a bibliography and it is to be regretted that room was not found for a complete citation of the references.

A word must be said in praise of the editors. In a book of this length some typographical errors are inevitable, but they are remarkably few and the layout throughout is clear and attractive.

In the face of so much research activity it may come as a surprise to the reader to learn that only three pathogens are marketed commercially and that microbial preparations account for less than 0.5 per cent of all insecticides sold. In spite of this and the fact that the tenor of the book is objective rather than propagandist, the contributors repeatedly express their belief in an important future for microbial methods of insect control. This book should

make a positive contribution to the attainment of their goal.

N. H. E. GIBSON

Lasers in Life Science

Laser Applications in Medicine and Biology. Edited by M. L. Wolbarsht. Pp. xiv+228. (Plenum: New York and London, 1971.) \$18.

THE subject of laser applications in medicine and biology has been of great interest to engineers and physicists in the laser industry because of their need to know how their technology can be applied. It is of equal interest to clinicians, constantly attempting to keep abreast of the latest procedures which may benefit their patients. Although laser technology is still advancing rapidly, some practical laser devices are being offered to a potential market where procedures for safety precautions, mode of operation, and potential applications are essentially unknown. A comprehensive treatise on medical applications of lasers is, therefore, a necessity. The book edited by M. L. Wolbarsht on this subject is timely and useful.

The topics which were included were thoughtfully selected and written by investigators who have pioneered in their fields. A preliminary discussion of laser characteristics and methods for measurement of energy or power output is fundamentally important to anyone contemplating the use of lasers. The reviews of explorations in the fields of tumour therapy, cell biology, and dentistry are historical accounts of the emergence of factual knowledge from a shroud of unfounded predictions of magical cures. The discovery of technical problems which burst the bubble of a hoped for cancer cure led to disillusionment and pessimism on the part of Riggle, Hoye, and Ketcham. The early predictions that lasers could drill out dental decay in a single flash proved to be totally impractical, but Stern found a prophylactic procedure which is showing great promise. Investigators who have used cell cultures and biochemical systems as test objects have reported a variety of fundamental laser effects which are proving useful to clinicians in designing more effective medical applications.

Wolbarsht assigned three chapters to descriptions of laser effects on the eye: (a) ocular damage from laser radiation; (b) safety considerations; and (c) ophthalmological applications. This is appropriate, for the eye is the most sensitive tissue to laser energy, and the application to ophthalmology is, to date, the only fully accepted clinical application of laser energy.

The final chapter on laser models outlines some parameters which suggest

guidelines for future research in laser biology. The newcomer to laser research should find this book a good reference, for it summarizes the principal concepts which have evolved within the past decade.

The problem with any book which attempts to describe events in a rapidly progressing technical field is the fact that it is outdated at the time of publication. Wolbarsht intends to meet this problem by keeping the reader abreast of recent developments, in subsequent volumes. This approach would also permit him to add or expand on subjects which, in retrospect, were either omitted or treated lightly in Volume I. Poorly developed subjects in the first volume were application of lasers in dermatology, specialized surgical procedures, and measurement of cellular parameters with laser instrumentation (for example, microprobe analysis and cell counting and sorting devices).

In summary, this book was prepared by qualified investigators who reviewed their subjects in a fair and factual manner. Although this treatise is reasonably comprehensive, there is room for expansion in future texts on this subject. DONALD E. ROUNDS

Glossy Archaeology

Science and Archaeology. Edited by Robert H. Brill. Pp. xi+288. (The Massachusetts Institute of Technology Press: Cambridge, Massachusetts, and London, August 1971.) £11.65; \$30.

ON reading the title of this volume one might presume it to be a textbook concerning the rapidly expanding field of physics and chemistry applied to art and archaeology. In fact it is an expanded version of the proceedings of a two-day symposium sponsored by the Division of History of Chemistry of the American Chemical Society in 1968 and comprises some twenty papers (with one exception by American authors) on very widely differing topics. For the most part the results outlined have been published elsewhere previously although not in such a magnificently produced volume; the text and illustrations are excellently laid out and this luxury is reflected in the price.

Although the meeting was "the Fourth Symposium on Archaeological Chemistry" there were also four papers on archaeological dating techniques including thermoluminescent and nuclear track dating. In the space allotted, only very superficial accounts could be given. In this respect it is worthwhile noting that the papers were given more than three years ago and not surprisingly great advances have taken place in many of the subjects. In particular work on thermoluminescent dating has made great strides during this

period and much of the paper on this subject is now out of date.

The seventeen papers devoted to chemical analysis of museum materials cover a number of aspects including outlines of new techniques. The fact that a museum object must go back to its showcase after chemical analysis without any significant damage means that special techniques must be devised. Such methods as X-ray fluorescence and neutron activation come into prominence and there are several examples of these techniques given. The majority of the papers, however, are concerned with particular applications of these techniques. These studies are concerned with attribution or authentication as well as examination of the techniques used in ancient crafts. The range of materials studied and discussed is surprisingly large; analyses of coins, jewellery, bronzes, silverware, ceramics, picture pigments, obsidian, bone and amber are described in one or more of the papers.

When one has looked through this book, one wonders who is likely to buy it for their shelves. It is not written for the specialist since none of the subjects is dealt with in any depth and for the general technical reader it by no means covers the whole field since the papers presented were chosen by the authors and do not fit any particular overall design. It is, however, a most elegant volume. E. T. HALL

Biochemical Technology

Advances in Biochemical Engineering. Vol. 1. Edited by T. K. Ghose and A. Fiechter. Pp. vii+194. (Springer Verlag: Berlin and New York, 1971.) 48DM; \$13.90.

THE series "Advances in Biochemical Engineering" is a very welcome addition to the literature, and I hope it will contribute to the development, to quote the editors, of "the yet to emerge hybrid discipline of biochemical engineering". There are possibly two approaches to be taken in the preparation of a series entitled "Advances in . . .", and these depend largely on the nature of the intended audience. The first involves "in depth" critical studies over restricted areas and the second is more generally discursive with the intention of bringing the newer developments to the attention of a wider audience. The first volume in the series falls primarily into the latter category. This by no means detracts from the value of the present volume but would, if continued throughout the series, reduce the potential of its contribution to biochemical engineering. Engineering studies seem to be less amenable to "popular" treatment than either industrial microbiology or even biochemical technology. Unfortunately,

the effect of such a presentation of biochemical engineering is to diminish the subject and to identify the potential engineering contribution with gadgetry and empiricism. Biochemical engineering must, if it is to deserve the title, develop into a systematic subject on a simple mathematical basis derived from the laws of conservation and the equations describing transport processes and physical biochemistry. Only in this way will the methodology be established of a rational and progressive discipline.

The first volume in the series covers a wide area including the fluid mechanical properties of fermentation fluids, the recovery of microbial mass from fermentation broths by flocculation and sedimentation, the kinetics of enzyme reactions involving solid substrates (cellulase-cellulose), enzyme production and utilization, regulation of both primary and secondary metabolite production and the use, particularly for microbial protein production, of gaseous, liquid and solid sources of carbon. As might be expected, the approach of the individual contributors varies with the nature of the particular topic. A survey style has been adopted to the properties of fluids and enzymes; the chapter on metabolite production is descriptive, while consideration of alternative carbon sources is in the form of a review. The chapters on the recovery of microbial mass and enzyme kinetics are largely mechanistic in the biochemical engineering sense.

The authors are to be congratulated on their presentations and for collecting a substantial amount of information, both qualitative and quantitative, on the various topics. The result is an easily readable text which will broaden the outlook of the workers in the area.

B. ATKINSON

Chemistry in the Cold

Low Temperature Spectroscopy. By Beat Meyer. Pp. xi+653. (Elsevier: Amsterdam; American Elsevier: New York, 1971.) Dfl.138; \$38.50; £15.70.

WITH the invention of the vacuum flask by Dewar a whole new field of low-temperature chemistry opened up. One important aspect of this has been the application of spectroscopic techniques, particularly when combined with the use of frozen solvents or matrices. Lewis and Porter saw the importance of photochemical processes of species in frozen organic glasses and Pimentel made a major advance by using frozen inert gases at 20 K as matrix materials. Such studies have concentrated on (a) primary photochemical processes—fluorescence, phosphorescence of isolated molecules including the perturbation of the guest energy levels by the host; (b) the

obtaining of spectra otherwise difficult to obtain, for example, Weltner's work on species of astrophysical interest; (c) the generation and examination of new unstable molecules, stabilized by a combination of low temperature and matrix isolation. The spectroscopic techniques include ultraviolet/visible, infrared (and very recently Raman) and electron spin resonance.

This book largely reflects the author's own interest in primary photochemistry. Indeed the preface states that "the book resulted from the author's need to have a text for undergraduates, PhD students and visiting scientists". Within these terms of reference the book succeeds admirably. For example, chapters 7 and 8 (111 pages altogether) provide a detailed description of apparatus and sample production; these chapters can be recommended to anyone contemplating entering the field. The earlier chapters in which are discussed the nature of spectra, photochemical processes and diffusion, however, are rather specialized. For example, chapter 4, "Details of Observed Spectra", is concerned exclusively with the effect of matrix on ultraviolet/visible spectra of isolated molecules—this ignores the considerable discussion in the literature of matrix effects on vibrational and electron spin resonance spectra. The book in fact contains very little about infrared and nothing about electron spin resonance spectra; this is a pity because such spectra have been of prime importance in establishing the generation of new species. There is also no mention of Rochkind's development of pseudo (or pulsed) matrix isolation, a technique of considerable analytical potential. Pages 233 to 493 provide a comprehensive summary of individual species up to July 1969 with some later references; this section, together with an extensive bibliography, is extremely valuable. Spectroscopic data recorded there are ultraviolet/visible and infrared which makes the absence of a discussion of infrared techniques more pointed.

Some specific points. The author is to be congratulated on providing so many illustrations, but just a few of them are rather misleading: for example, Fig. 3.2 does not match its caption; Fig. 8.6 (a), (b) and (c) do not match the text, and there is no Fig. 8 (d) at all; the scale drawings of matrix materials in Fig. 6.4 do not agree with the lattice parameters in Table 8.2. There are few omissions and misprints—the reviewer only noticed the following: in the equation on page 25 the terms are not properly defined; the data for SnS in Table 3.2 cannot match the equation on page 27; on page 290 HNS should be HNSi.

In short, this is a good book for a limited audience. J. J. TURNER

CORRESPONDENCE

Geological Abstracts

SIR,—The article, "Abstracts Abandoned" (*Nature*, **234**, 167; 1971), contains sufficient contradictions and confusion to require comment. The US Geological Survey publications that will be suspended at the end of 1971 are not providing, as indicated by their titles (*Abstracts of North American Geology* and *Geophysical Abstracts*), comprehensive bibliographic coverage of the literature but only about 30 per cent of it. The Geological Society of America's *Bibliography and Index of Geology*, a product of the American Geological Institute's GEO-REF system, publishes over double that amount of deeply indexed and annotated citations in twelve monthly issues plus an annual cumulative author and subject index under hard covers. This was a major consideration in the Survey's final decision to suspend publication in favour of one English-language bibliography that would contain references to the world literature rather than parochial coverage by discipline, mission, or territorial division. Moreover, in conversations between the Survey and AGI prior to the decision, it was agreed that GEO-REF and the GSA Bibliography would increase their coverage at least to include all of the North American and geophysical material heretofore contained in the suspending publications.

The \$10 a year price of the *Abstracts of North American Geology* was completely artificial as anyone producing bibliographies must realize. It was originally based on the cost of a print overrun only, beyond the requirements of the Survey's internal free distribution. As such, it was cheaper than Professor Clayton's *Geographical Abstracts* and Dr Howie's *Mineralogical Abstracts*. Had it been priced realistically, it might still be publishing.

As to the \$250 price tag on the *Bibliography and Index of Geology*, it is too bad that the geological community has never accepted the value of information as have the chemists, biologists, and physicists. This is particularly unfortunate as the geologists' use of their archival information is much greater than the use made in other disciplines. They may have to accept it in the future or do without when State funding agencies run out of money or patience, or both. In any event, the *Bibliography* is less expensive than the *Bibliographie des Sciences de la Terre*, the only comparable geo-

logical publication in the western world, and cheaper by an order of magnitude than the chemical, biological, and physical counterparts.

We, at GEO-REF, work very closely with both the Bureau de Recherches Géologiques et Minières and the Centre National de la Recherche Scientifique, the producers of the *Bibliographie des Sciences de la Terre* and the *Bulletin Signalétique* and have great respect for their publications. Nevertheless, I fail to see the compensation that the combined French-language service will provide for English-speaking geologists. They do not (and will not so far as I know) even satisfy Professor Clayton's passion for abstracts. We are also receiving cooperation in our bibliographic work from CSIRO in Australia and have plans for input from Europe, including an exchange with BRGM and CNRS. We would welcome a contribution from the UK, but it would have to be of better quality than one we received from there in 1967 and 1968.

Finally, may I call to your attention that the GEO-REF bibliographic service not only produces the printed *Bibliography and Index of Geology*, but it is the only comprehensive service in geology (in any language) that is distributing tapes to data centres, libraries, and universities for computer searching. We are also providing the annual indexes for a dozen primary journals, producing ten volume cumulative indexes for certain serials, and are providing the material for special topical bibliographies such as the *Bibliography of Coal in Kentucky*, published by the Geological Survey of Kentucky, and the *Bibliography and Index of Micropaleontology* which is being published monthly by the American Museum of Natural History. We are seeking further uses of our tape file and are formulating plans for inputting the historical data of geological literature starting in the mid-nineteenth century.

Yours faithfully,

JOEL J. LLOYD

American Geological Institute,
2201 M Street NW,
Washington DC 20037

Heads in Bags!

SIR,—I am shocked that when commenting on the Compton inquiry about alleged British Army brutality in Northern Ireland, you do not mention

as much as a single one of the many horrible cases of terrorism with which the British soldiers have been confronted. You go into considerable detail describing individual cases of maltreatment at the hands of British soldiers, whereas you pass off merely as "growing violence" wholesale murder and arson.

It is of course deplorable that people have been maltreated, and some of them probably unjustly so, by British Army personnel. What I object to is that you apparently worry more about Mr Anderson's heart condition, which, as mentioned by you, may have become worse as a result of the treatment given him, than you worry about the heart conditions of the mourning relatives of murdered British soldiers! You are also concerned about the psychological effects of putting a bag over Mr Campbell's head, but not about the effects on wives and children who have seen husbands and fathers murdered in full view of them! You mention the "kado" grip used on Mr Joseph, but not how the wives and children just mentioned were thrown out in the streets, so that their homes also could be blown up! Finally, is it worse to be hit by a rubber bullet, as Mr Gilmore was, than by broken glass and flying bricks in a bomb explosion?

And you, Sir, blame the Compton committee for pedantry!

The thing that must surprise any person who has not his "head in a bag" is that the British Army has been capable of reacting with such restraint in Northern Ireland. For whatever injustices may have been done to the other side in former times, and I don't excuse these, they cannot possibly justify the present terrorism. Certainly your own point of view ought to imply that the other side also should abstain from physical and psychological violence. Or perhaps the other side should not be bound by the same rules? Personally I think that an equivalent to the Compton inquiry into the methods of the other side is far more urgently required than a new one by Lord Parker into the methods used by the British Army.

How does it come, Sir, that you don't suggest that?

Yours faithfully,

HARALD TREFAILL

Fysisk Institutt Avd. B,
Universitetet i Bergen

Announcements

University News

Dr Roy Ellis, St Bartholomew's Hospital Medical College, London, has been appointed professor and head of the Department of Medical Physics, University of Leeds.

Appointments

Dr L. E. Glynn, deputy director of the MRC Rheumatism Research Unit, has been appointed director of the **Mathilda and Terence Kennedy Institute of Rheumatology**.

Professor M. Freedman, University of Oxford, **Professor A. T. Peacock**, University of York, and **Professor B. E. Supple**, University of Sussex, have been appointed members of the **Social Science Research Council**.

New members of the **National Electronics Council** include **Mr R. W. Wright**, National Economic Development Council, **Mr A. A. Dyson**, Institution of Electronic and Radio Engineers, and **HRH the Duke of Kent**.

Miscellaneous

The **1972 Bertner Foundation award** for outstanding achievements in cancer research will be made to **Dr Howard Temin**, University of Wisconsin.

Lord Kearton, chairman of Courtaulds Limited, has been elected president of the **Society of Chemical Industry** for 1972-73, in succession to **Mr G. H. Beeby**.

Dr Kenneth Mellanby, director of Monk's Wood Experimental Station, has been elected president of the **Institute of**

Biology for 1972. Other newly elected officers and members of council include **Dr H. O. J. Collier**, **Dr T. G. Onions** (vice-presidents), **P. N. O'Donoghue** (honorary secretary), **Dr G. E. Beedham**, **Dr D. C. Breeze**, **Dr M. Cohen**, **J. Forsyth** and **W. H. Parry** (ordinary members).

Applications are invited for the prize of 500,000 Belgian francs to be awarded by the **Fondation de Physiopathologie Professeur Lucien Dautrebande**, Liège, for outstanding work on human or animal physiopathology which has therapeutic implications. Further information on the prize can be obtained from the office of the Foundation, 35 Chaussée de Liège, 5200 Huy, Belgium.

Submissions are invited by the Council of Engineering Institutions for the **1972 MacRobert Award**, which consists of £25,000 and a gold medal. The prize will be awarded to a team of no more than five people or to an individual making an outstanding contribution to engineering, the physical technologies or to the application of the physical sciences. Further information can be obtained from the MacRobert Award Office, CEL, 2 Little Smith Street, London SW1P 3DL.

Errata

In the article "NAD in the Metabolism of Motile Spermatozoa" by D. E. Brooks and T. Mann (*Nature*, 234, 301; 1971), the labelling on the vertical axis of Fig. 1 should read

$$\frac{\text{NAD}^+}{\text{NAD}^+ + \text{NADH}} \times 100$$

In the article "Temperature Changes associated with Adiabatic Decompression in Geological Processes" by Hans Ram-

berg (*Nature*, 234, 539; 1971), equations (6) and (7) should read:

$$\left(\frac{dT}{dh}\right)_H = -\frac{V\rho g}{C_p} \quad (6)$$

$$\left(\frac{dT}{dh}\right)_H = -\frac{T\alpha V\rho g}{C_p} \quad (7)$$

In the article "New Theory for Giant Loops" by John C. Brandt and Stephen P. Maran (*Nature*, 235, 38; 1972), the following corrections should be made: Lines 4-8 on page 39 should read "Zuzak¹¹ solves the energy problem by postulating a continuing energy supply for the supernova remnant model of a giant loop, namely cosmic ray particles accelerated by a pulsar or other source at the centre"; lines 33-35 on page 39 should read "At 10⁴ K, the sound speed is about 10 km s⁻¹. A hydromagnetic shock associated with the expansion should travel at a somewhat greater velocity".

International Meetings

February 16, **Semiconductor Detectors in Clinical Investigation and Research**, London (The General Secretary, British Institute of Radiology, 32 Welbeck Street, London W1M 7PG).

February 17, **The Use of Enzymes in Analysis**, Manchester (Society for Analytical Chemistry, 9-10 Savile Row, London W1X 1AF).

February 23, **Anaesthetic and Neuromuscular Blocking Drugs**, London (Dr J. F. Cavalla, John Wyeth and Brother Ltd, Huntercombe Lane South, Taplow, Maidenhead, Berkshire).

March 5-10, **Analytical Chemistry and Applied Spectroscopy**, Cleveland (Harry Frazer, Fisher Scientific Company, 585 Alpha Drive, Pittsburgh, Pennsylvania 15238, USA).

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